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THE EFFECT OF BANDAGING OF THE LEGS ON THE RATE OF BLOOD FLOW IN THE FEET*

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It has been suggested by some observers of the condition known as "trench-foot," which was so common among the soldiers in the trenches in Belgium and France, especially during the first winter of the war, that obstruction of the venous return by the puttees worn by the British troops was an important contributory factor.

The observations which form the subject of this paper are concerned only with the effect of relatively short applications of the puttees. Having been made in a laboratory on a normal man, they do not reproduce what is probably an important condition for the development of trench-foot, if the pressure of the puttees has anything to do with the condition, namely, the swelling under an already tight, wet, and dirty bandage. So far as the technic of the observations is concerned, it would have been easy enough to study the circulatory changes at first hand on soldiers. Not having been able to do this (and of course in such matters the military authorities must be the final judges as to what military exigencies will allow), the writer is, for the present at least, obliged to content himself with publishing a few specimen results on a normal man, which he had hoped to compare with data obtained by clinical studies of the actual condition at different stages. Since, however, so far as I am aware, no investigation of the influence of such bandages applied to the legs on the flow of blood in the feet has been published, and since such measurements have an interest in other relations, the application of bandages to limbs being so common in surgery and in certain medical procedures, it seems worth while to record the result of this preliminary study.

The observations were made on M. C., a healthy man, aged 26 years, weight 165 pounds, height 5 feet, 10 inches. Numerous measurements of the blood flow in his hands and feet have been made in the past few years, so that the range of variation of the flow under the

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conditions of the observations is well known.[1] In all the experiments the amount of water in the calorimeters was 2,550 c.c. The puttees were always applied over the trousers, which were tucked in in the usual way.

October 22. M. C., four hours after breakfast, put his feet in the bath at 11:02 and in calorimeters at 11:18 a. m. The pulse was 90. At 11:40 a puttee was put on the right leg with the ordinary degree of firmness. At

TABLE 1

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
11:17	31.08	31.22		12:05	31.705	31.50	24.1
11:20	31.19	31.24	24.3	12:07	31.82	31.63	
11:22	31.24	31.27	24.3	12:09	31.85	31.65	24.1
11:24	31.28	31.29	24.3	12:11	31.88	31.675	
11:26	31.32	31.30		12:13	31.91	31.69	24.1
11:28	31.36	31.34	24.4	12:15	31.96	31.74	
11:30	31.395	31.36		12:17	32.00	31.775	24.1
11:32	31.41	31.37	24.2	12:19	32.06	31.83	
11:36	31.44	31.39	24.2	12:21	32.10	31.86	24.0
11:38	31.47	31.40	24.3	12:23	32.14	31.90	
11:40	31.495	31.43		12:25	32.19	31.94	24.1
11:43	31.52	31.44	24.2	12:27	32.23	31.97	
11:45	31.53	31.45		12:29	32.295	32.04	24.1
11:47	31.55	31.455	24.2	12:31	32.34	32.08	
11:49	31.58	31.46	24.2	12:33	32.39	32.125	24.1
11:51	31.60	31.465		12:35	32.43	32.16	
11:53	31.62	31.47	24.2	12:38	32.47	32.18	24.1
11:55	31.63	31.475		12:40	32.49	32.195	
11:57	31.66	31.485	24.2	12:42	32.50	32.22	24.1
11:59	31.68	31.50		12:44	32.52	32.24	
12:01	31.72	31.54	24.2	12:46	32.44	32.19	
12:03*	31.76	31.57		1:01	32.12	31.87	

* Right foot feels more comfortable than left, and a little warmer.

12:23 the puttee was rapidly taken off. At 12:44 the feet were removed from the calorimeters.

The cooling of the calorimeters in fifteen minutes was 0.32 C. The volume of the right foot was 1,171 c.c., of the left 1,128 c.c. The water equivalent of the calorimeters with their contents was, right 3,616 c.c., left 3,584 c.c. The rectal temperature was 36.89 C.

In Table 1 is given an experiment in which a puttee was applied only to the right leg. Since the pressure is not the only effect which

1. Stewart, G. N.: Jour. Exper. Med., 18, 354, 372.

the bandage can exert on the blood flow, but the warmth may also be important, in other experiments puttees were applied to both legs, but with different degrees of tightness.

In this experiment the flow in the feet was less than in the others cited, possibly because the subject had been going about for some time with bare feet and legs, completing the preparations for the observations; probably also in part because the experiment was made several hours after a meal. The taking of food, since it increases metabolism, must also increase the heat loss, and therefore the vasodilatation of the skin. The initial vasoconstriction due to exposure of the feet

TABLE 2

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
2:37	30.89	30.95		3:10	32.95	32.875	
2:40	30.99	31.08	24.0	3:12	33.01	32.95	24.0
2:42	31.20	31.20	24.1	3:14	33.09	33.06	24.1
2:44	31.40	31.39	24.2	3:16	33.18	33.13	
2:46	31.57	31.55	24.3	3:18	33.26	33.19	24.1
2:48	31.73	31.71	24.3	3:20	33.36	33.30	
2:50	31.89	31.86		3:22	33.45	33.37	24.1
2:52	32.02	32.99		3:24	33.53	33.46	
2:56	32.24	32.18	24.1	3:26	33.61	33.55	24.1
2:58	32.33	32.28	24.1	3:28	33.70	33.65	
3:00	32.45	32.38		3:30	33.78	33.72	24.15
3:02	32.58	32.51	24.0	3:32	33.86	33.785	
3:04	32.72	32.65		3:34	33.81	33.74	
3:06	32.80	32.74	24.0	3:46	33.50	33.43	
3:08	32.88	32.80	24.0				

tended to pass off as the experiment proceeded, the rate of flow increasing in the successive periods. But there was no indication that the puttee on the right leg in any way interfered with the flow in the right foot. The ratio of the minute flow in the left foot to that in the right was almost precisely the same in the eighteen minutes before the application of the puttee as in the forty minutes during which it remained on. The data are displayed in succinct form in Table 5.

In the experiment shown in Table 2 a puttee was applied firmly to the right leg. The degree of pressure of the puttee was about what the soldier commonly employs. On the left leg a similar puttee was applied quite loosely, so that the heat-conserving effect would be the

same as on the right leg, while the pressure was negligible. For the ten minutes before the application of the puttees the flow per minute per 100 c.c. of part was 7.18 gm. for the right and 7.2 gm. for the left foot, practical equality. For twenty minutes with the puttees on, the flow was 6.43 gm. and 6.58 gm. per minute per 100 c.c. For fourteen minutes after removal of the puttees the flow was 8.09 gm. and 8.13 gm. per 100 c.c. per minute for the right and left foot respectively.

TABLE 3

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
2:36	30.87	30.88		3:17	33.33	33.22	24.2
2:38	30.93	30.97	24.1	3:19	33.40	33.27	
2:40	31.09	31.09	24.1	3:21	33.47	33.34	24.2
2:42	31.28	31.27		3:23	33.52	33.42	
2:44	31.42	31.43	24.1	3:25	33.595	33.47	24.2*
2:46	31.56	31.55		3:27	33.65	33.54	24.25
2:48	31.70	31.68	24.0	3:29	33.72	33.59	
2:50	31.85	31.82		3:31	33.78	33.66	24.2
2:52	31.99	31.92	24.1	3:33	33.83	33.72	
2:54	32.12	32.055		3:35	33.90	33.79	24.2
2:56	32.25	32.16	24.1	3:37	33.97	33.86	
3:00	32.41	32.34	24.3	3:39	34.02	33.915	24.2
3:02	32.53	32.45	24.25	3:41	34.07	33.96	
3:05	32.70	32.60	24.3	3:43	34.13	34.02	24.2
3:07	32.805	32.69		3:45	34.19	34.07	
3:09	32.90	32.80	24.25	3:47	34.26	34.14	24.2
3:11	33.00	32.91		3:49	34.30	34.18	
3:13	33.11	33.03	24.2	3:51	34.52	34.12	
3:15	33.22	33.13		4:05	33.90	33.76	

* Left leg felt cool after the puttee was taken off it; the right leg felt warm.

October 26. M. C., had lunch one hour before, and had walked outside for some time. The pulse was 94. The feet were put in the bath at 2:22, and in calorimeters at 2:39 p. m. At 2:52 a puttee was put firmly on the right leg and one slackly on the left leg. At 3:16 the puttees were taken off quickly. At 3:32 the feet were taken out of the calorimeters.

The cooling of the calorimeters in twelve minutes was 0.31 C. The volume of the right foot was 1,180 c.c., of the left 1,128 c.c. The water equivalent of the calorimeters with their contents was, right 3,623 c.c., left 3,385 c.c. The rectal temperature was 37.05 C.

If the twenty minute period is analyzed, it is seen that for the first half of it the flow was 6.84 gm. and 6.97 gm. for the two feet,

respectively; and for the second half 6.13 gm. and 6.3 gm. The diminution in the right foot is not due to pressure of the bandage, since it is the same as the diminution in the left. Removal of the puttees was followed by some increase in flow in both feet, but without disturbance of the relation of equality in the two feet present from the beginning of the experiment. The increased flow cannot be interpreted as due to the vasomotor paralysis following the removal of a tight bandage described by Bier. For the puttee on the right leg was not so tight as to occasion the slightest discomfort, and the increased flow was present in the left foot as well as in the right.

TABLE 4

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
11:26	30.92	30.78		12:05	32.46	31.99	24.15
11:29	31.00	30.86	25.2	12:07*	32.56	32.08	24.2
11:31	31.07	30.92	25.2	12:09	32.61	32.14	24.2
11:33	31.17	30.98	25.2	12:11	32.68	32.18	
11:35	31.27	31.07	25.1	12:13	32.73	32.23	24.2
11:37	31.37	31.16	24.7	12:15	32.80	32.28	24.25
11:39	31.49	31.26	24.4	12:17	32.88	32.34	
11:41	31.59	31.34		12:19	32.96	32.40	24.2
11:45	31.71	31.48	24.5	12:21	33.00	32.455	
11:47	31.76	31.45	24.4	12:23	33.07	32.51	24.2
11:49	31.81	31.48	24.3	12:25	33.09	32.55	
11:51	31.895	31.55		12:27	33.13	32.59	24.1
11:53	31.96	31.59	24.1	12:29	33.17	32.64	24.1
11:55	32.04	31.65	24.0	12:31	33.19	32.66	
11:57	32.16	31.755	24.0	12:33	33.20	32.68	24.0
11:59	32.22	31.81	24.0	12:35	33.20	32.68	
12:01	32.28	31.85	24.05	12:37	33.12	32.615	
12:03	32.37	31.91	24.1	12:40	32.81	32.31	

* The right leg was comfortable, the left somewhat tired and uncomfortable.

In the experiment shown in Table 3 the procedure was the same as in the last experiment, except that the puttee on the left leg (put on quite slack) was removed some time before the other, in order to see whether the increased loss of heat from the left leg caused any effect on the flow in the right foot. There was, as a matter of fact, a slight diminution in the flow in the left foot, which might be attributed to vasoconstriction due to increased cooling. If this was the cause,

the action extended reflexly to the right limb, the puttee on which had not been disturbed, as the ratio of the flows in the two feet remained unchanged. There was no indication that the pressure of the puttee on the right leg diminished the flow in the right foot.

October 29. M. C., one hour after lunch, put his feet in the bath at 2:20, in the calorimeters at 2:37 p. m. The pulse was 88. At 2:56 a puttee was

TABLE 5

Date	Pulse Rate	Temperature (C.) of				Volume of Foot in C.c.		Heat Given Off in Gm.—Calories		
		Room	Arterial Blood	Calorimeters						
				Right	Left	Right	Left	Right	Left	No. Min.
10/22	90	24.3	36.29	31.27	31.25	1,171	1,128	2,115	1,792	18
		24.1	.....	21.83	31.67	.....	.....	5,170	4,551	40
		24.1	.....	32.36	32.00	.....	.....	2,680	2,580	19
10/26	94	24.2	36.45	31.61	31.60	1,180	1,128	3,695	3,549	10
		24.05	.....	32.52	32.46	.....	.....	2,855	2,825	10
		24.05	.....	32.99	32.94	.....	.....	2,253	2,248	10
		24.1	.....	33.56	33.49	.....	.....	3,478	3,423	14
10/29	88	24.1	36.36	31.92	31.86	1,190	1,133	3,158	2,913	10
		24.25	.....	32.87	32.78	.....	.....	4,720	4,530	17
		24.2	.....	33.56	33.43	.....	.....	2,051	2,027	10
		24.2	.....	34.04	33.92	.....	.....	3,557	3,516	18
11/ 2	80	24.8	36.24	31.38	31.16	1,171	1,142	2,133	1,905	8
		24.3	.....	31.88	31.54	.....	.....	2,007	1,600	10
		24.1	.....	32.36	31.92	.....	.....	3,743	3,290	16
		24.2	.....	32.90	32.37	.....	.....	2,155	1,923	10
		24.1	.....	33.14	32.60	.....	.....	1,610	1,725	12
11/30	100	20.5	36.41	31.19	31.05	1,171	1,142	6,183	4,602	20
		20.6	.....	31.40	31.23	.....	.....	3,073	2,294	10
		20.9	.....	32.19	31.74	.....	.....	2,759	2,459	10
		21.1	.....	32.60	32.10	.....	.....	1,797	1,675	6
		21.2	.....	33.13	32.57	.....	.....	3,952	3,703	18
		21.0	.....	33.53	32.92	.....	.....	1,121	1,064	6

put firmly on the right leg and one slackly on the left leg. At 3:17 the puttee was rapidly taken off the left leg; at 3:29 off the right leg. The feet were taken out of the calorimeters at 3:49.

The cooling of the calorimeters in fourteen minutes was 0.36 C. The volume of the right foot was 1,190 c.c., of the left 1,133 c.c. The water equivalent of the calorimeters with their contents was, right 3,630 c., left 3,588 c.c. The rectal temperature was 36.96 C.

Indeed, with both puttees on, the flow was slightly and equally increased in both feet, probably on account of the heating effect.

In the next experiment to be quoted (Table 4) the puttee was purposely put on the left leg so tightly as to produce a certain amount of discomfort, while it was applied to the right leg with the ordinary, comfortable degree of pressure. The subject was hungry (four and

TABLE 5—*(Continued)*

Blood Flow in Gm. per Min.		Flow per 100 C.c. of Foot per Min.		Ratio of Flow in 2 Feet	Remarks
Right	Left	Right	Left		
26.58	22.39	2.26	1.96	1:1.14	Before puttee was put on
32.20	27.37	2.75	2.42	1:1.14	Puttee on right leg
39.88	35.23	3.40	3.12	1:1.09	Puttee off
84.82	81.31	7.18	7.20	1:1.00	Before puttees put on; after eating
80.72	78.67	6.84	6.97	1:1.02	Put. R. firm, L. slack (first 10 minutes)
72.35	71.16	6.13	6.30	1:1.03	Put. R. firm, L. slack (next 10 minutes)
95.51	91.78	8.09	8.13	1:1.00	Puttees off
79.08	71.92	6.64	6.34	1:1.05	Before puttees put on; after eating
88.39	82.52	7.42	7.28	1:1.02	Put. R. firm, L. slack
81.39	76.87	6.83	6.78	1:1.01	Puttee off L. leg
94.64	88.94	7.95	7.85	1:1.01	Puttees off both legs
59.73	51.08	5.10	4.47	1:1.14	Before puttees; after eating
50.00	37.03	4.27	3.24	1:1.31	Put. R. firm, L. too tight (first 10 minutes)
65.21	51.09	5.57	4.52	1:1.23	Put. R. firm, L. too tight (next 16 minutes)
69.60	58.82	5.94	4.71	1:1.26	First 10 minutes after puttees removed
46.56	42.70	3.97	3.73	1:1 06	Next 12 minutes after puttees removed
65.80	47.09	5.62	4.17	1:1.34	Before puttees put on
60.29	51.35	5.92	4.49	1:1.31	Last 10 minutes before puttees
72.64	58.51	6.20	5.12	1:1.21	First 10 minutes with puttees
87.34	71.97	7.46	6.30	1:1.16	Next 6 minutes with puttees
74.27	59.52	6.35	5.21	1:1.21	After tightening tapes on R. leg
54.06	42.34	4.61	3.70	1:1.24	After puttees off

one-half hours after breakfast) and the flow in the feet was less than in the experiments shown in Tables 2 and 3. The flow in the two feet was 5.10 gm. and 4.47 gm., respectively, per 100 c.c. per minute for an eight minute period before the application of the bandages (ratio of left to right 1 to 1.14). For a period of twenty-six minutes with the puttees on the flow was 5.13 gm. and 4.04 gm., respectively

(ratio 1 to 1.27). The cutting down of the flow by the improperly applied puttee on the left leg is evident, although the bandage was not put on so tight as to cause an extreme degree of discomfort.

November 2. M. C., four hours after breakfast, put his feet in the bath at 11:07, in the calorimeters at 11:27:30 a. m. At 11:41 putees were put firmly on both legs, but the left was much tighter and less comfortable than the right. At 12:11 the puttees were rapidly taken off. The feet were taken out of the calorimeters at 12:35.

The cooling of the calorimeters in twelve minutes was, right 0.31 C., left 0.305 C. The volume of the right foot was 1,171 c.c., of the left 1,142 c.c. The water equivalent of the calorimeters with their contents was, right 3,616 c.c., left 3,595 c.c. The pulse was 80. Rectal temperature was 36.96 C.

If the period is analyzed, the deficit in the left foot is seen to be greatest in the first ten minutes (flow in right foot 4.27 gm. per 100 c.c. per minute, in left 3.24 gm., giving a ratio of 1 to 1.31). The flow in the right foot is also diminished somewhat. In the remaining sixteen minutes of the period of application of the puttees the flow increases in both feet, but relatively more in the left, as the venous pressure rises and forces the block (5.57 gm. for right and 4.52 gm. for left foot, ratio 1 to 1.23). Even with the tight bandage on the left leg, the initial flow in the left foot is soon reestablished. This, however, is in a healthy man, whose cutaneous flow is uniformly good. It seems clear enough that in men who habitually suffer from cold feet, and whose foot flow is therefore normally small, the injudicious application of a puttee might easily cause a harmful reduction in the flow which would render the foot more readily susceptible to the effects of cold and wet, of slight injuries and of the passive congestion associated with standing for long periods in one position. On the other hand, so far as can be judged from experiments of such relatively short duration, a properly adjusted puttee not only causes, under normal conditions, no permanent diminution in the foot flow, but may even somewhat increase the flow, probably largely because of its heat conserving property.

In another experiment (that of November 30) different methods of fastening the puttees were investigated. That on the right leg was tied by tapes an inch wide, that on the left leg was fastened smoothly by a safety pin. The tapes caused a somewhat tighter feeling, according to the subject, than the pin, but both legs felt comfortable. After the first sixteen minutes the tapes were made considerably tighter on the right leg, but not uncomfortably so. As will be seen in Table 5, the tightening of the tapes did not produce any diminution in the flow in the right foot as compared with that in the left, although the flow in both was now diminishing, and diminished still more after removal of the puttees. This last decrease in the flow, seen also in the experiment of November 2, may be due to a vasoconstriction associated with

cooling of the limbs after removal of the bandages. However, a terminal vasoconstriction due to the exposure is not uncommonly seen in long experiments on the foot flow when no bandages have been applied.

SUMMARY

It is shown that a puttee applied with the usual degree of pressure on the leg causes no diminution in the blood flow through the foot. When it is put on so tight that some discomfort is produced the flow in the foot is at first reduced.

THE BILE CONTENT OF THE BLOOD IN PERNICIOUS ANEMIA *

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The lemon yellow color of the skin in pernicious anemia has always been closely associated with the idea of jaundice and is often referred to as an icteroid hue. But the almost invariable absence of bile from the urine has stood in the way of our calling pernicious anemia patients definitely jaundiced. The frequent appearance of urobilin in the urine has suggested an explanation for the color of the skin, and we frequently see the term "urobilin icterus" in this connection.

Hemolysis, which is the most definite pathologic entity of pernicious anemia, is also fundamentally associated with the symptom jaundice and we are therefore enticed by the term "hemolytic jaundice" in pernicious anemia.

Just what relation exists between the appearance of bile pigments in the blood and the yellow pigmentation of the skin in pernicious anemia has been the object of this investigation.

The presence of bile in the blood in any condition, physiologic or pathologic, has not been extensively investigated, and satisfactory methods have not been available. Rather extensive chemical studies of the blood in pernicious anemia have been made, especially by Erben,[1] Rumpf[2] and von Jakchs,[3] but these and others make no mention of biliary elements save that the amounts of cholesterin and lecithin are diminished; a finding that is common to many chronic diseases, especially those in which starvation is a factor. Sylaba[4] found bilirubin in the blood in eight cases of pernicious anemia. Wilbur and Addis,[5] in their observations on urobilin in the plasma, did not find that substance present in pernicious anemia.

METHODS

Oxalated plasma was used in every case. Gmelin's test for bilirubin was used in testing for bile pigment, which gives the well-known blue-green color with nitric acid. This method was applied to the

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1. Erben: Ztschr. f. klin. Med., 1900, **40**, 266.
2. Rumpf: Berl. klin. Wchnschr., 1901, **38**, 477.
3. Von Jakchs: Klinische Pathologie des Blutes, Jena, 1896, p. 311.
4. Sylaba: Abst. in Folia Hematol., 1904, **1**, 283, 589.
5. Wilbur and Addis: THE ARCHIVES INT. MED., 1914, **13**, 235.

plasma by Gilbert,[6] who arrived at conclusions as to the normal bilirubin content of the human plasma. Gmelin's test on the plasma is made by putting nitric acid under the plasma in a test tube, with a small pipet. A white coagulum is formed at once at the junction, which soon develops into a white layer of a certain thickness, and remains fairly constant as the acid dissolves the coagulum at the lower border and forms it at the upper, thus ascending through the plasma. The blue-green color develops in a line at the midst of the white zone and remains in the same relative position as long as there is any white zone. When bilirubin is present in small amounts, the blue color may be as long as a half hour in appearing. Since the white coagulum forms a good background on which to see the blue line, the tests are very satisfactory and give unequivocal results.

In a later series of observations, part of which is included in this report, a few specimens were examined for bilirubin by the Huppert-Cole method and out of twenty giving a positive Gmelin test all save one gave a positive Huppert-Cole in addition. The exceptional case, however, gave but a very faint Gmelin test. Included in this series of twenty are eight cases of pernicious anemia, six of which gave a positive Huppert-Cole test as well as a positive Gmelin, the remaining two being negative by both methods. It is the impression of the writer that the Huppert-Cole test is not so delicate as Gmelin's test.

Just what minimum amount of bilirubin gives Gmelin's test is not definitely known, but ordinary gallbladder bile can be diluted 600 times in colorless plasma and still give this test. Gallbladder bile contains about 2 per cent. pigment, chiefly bilirubin, so approximately the test will show one part in 30,000. But it is quite certain that a plasma may be visibly bile stained and still not give Gmelin's test, for ordinary gallbladder bile can be diluted 5,000 times and a yellow color be just perceptible in a column 1 cm. deep, and since only 2 per cent. of bile is bilirubin, we conclude that bilirubin can be diluted 250,000 times and still be just visible, making inspection of the plasma a test much more sensitive than Gmelin's.

There is no way of concentrating small amounts of pigment, nor is there any very satisfactory quantitative method for measuring bilirubin. The writer has accepted the staining of the plasma as a measure of bilirubin content, for no other yellow substance was found in any plasmas, and the intensity of Gmelin's test is proportional to the degree of yellow staining.

Lutein is said to stain the plasma yellow, as well as to give a blue-green color with nitric acid. Luetin can be differentiated from bilirubin in that it is not separated from its solution in chloroform by

6. Gilbert: Compt. rend. Soc. de Biol., 1905, 1906.

water containing an alkali (Hammarsten). A characteristic spectrum is also described for it. We have never been able to identify luetin in the plasma in any instance, in particular in one faintly stained plasma which gave a positive Gmelin's test, but not the Huppert-Cole test.

For the purpose of recording, the bilirubin content of the blood is expressed by the dilution required to diminish the staining to a point where it is just perceptible in a column 1 cm. deep. Plasma to be thus measured must be obtained free from hemolysis. In diluting the plasma with distilled water, a faint precipitate of globulin usually appears in suspension, which can be put in solution by a drop of ammonium hydroxid. The end point of the dilution, that is, where the yellow color is just perceptible, is found by comparing the solution in a small test tube with a similar column of distilled water. To facilitate reading, the tubes are held parallel and observation made down the length of the tubes, and a device which serves to bring the two columns to still more equal terms is to immerse the ends of the tubes in an inch or two of water in an evaporating dish. This cuts out all reflecting surfaces and gives a white background. While this by no means serves to make quantitative estimates of bilirubin in the blood, it does give a method for comparing and recording the jaundice of the plasma within about a 10 per cent. error. Specimens of plasma examined by this method showed a staining that could be diluted as high as 275 times. The lesser degrees of staining are not so satisfactorily estimated and no measurement under ten is very reliable. All at twenty or over gave a positive Gmelin's test.

Quite recently there has appeared a method[7] for measuring bilirubin in plasma, but as yet no general use has been made of it.

For urobilin Ehrlich's aldehyde test was employed on oxalate plasma, applied directly. Most of the blood protein is first precipitated by one or two volumes of saturated alcoholic solution of zinc acetate and centrifugalizing; 5 to 10 drops of the aldehyde reagent are added and a color from pink to dark red develops, which gives the characteristic spectrum if urobilin or urobilinogen is present. While negative results by this method are not always acceptable the writer has found the test positive in three instances and then strongly positive.

For detection of bile salts the Pettenkofer test was employed. The Pettenkofer test is not specific for bile salts or for any group and therefore all its results must be carefully criticised. On account of its lack of specificity the Pettenkofer reaction appears very seldom in the literature (other than biochemical). Pettenkofer, in his original description, named forty substances that give it. Of those that occur in the blood and are known to give it are cholesterin and lecithin;

7. Hooper and Whipple: Am. Jour. Physiol., 1916, **40**, 332.

also many alcohols, fatty acids and aromatic substances. The absorption spectrum of the Pettenkofer test is said to be more nearly specific than the color test alone, so we have controlled all our color tests by the spectroscopic examination in addition.

The Pettenkofer test cannot be applied directly to the blood or blood plasma, but the bile salts must be separated from the blood proteins and be relatively free from lecithin and cholesterin. This has been done by precipitating the salts with basic lead acetate and ammonium, after the proteins have been removed with large amounts of alcohol—the Hoppe-Seyler method—a process which is long and tedious, requiring much time and many manipulations. But it was found that the bile salts can be obtained relatively free by dialyzing the plasma through collodion sacs into water or water and alcohol.

This method has been employed in all cases. Five c.c. of plasma are dialyzed into from 5 to 15 c.c. of water or equal parts water and alcohol, and two or three such dialyzers are allowed to stand over night. The collected dialysate is then concentrated and the Pettenkofer test applied, and the spectroscopic examination made, if any color develops. For the test 2 c.c. of concentrated dialysate is placed in a small flask with 2 or 3 drops of a 1 to 1,000 aqueous solution of furfurol, and 2 c.c. of concentrated sulphuric acid is added drop by drop from a pipet; but the contents of the flask are kept at 60 C. by immersing the flask in a bath at 60 C. and keeping the mixture agitated. When the acid is added too rapidly a dark, reddish-brown color sometimes appears from the charring action of the acid on the small amounts of protein or sugar that dialyze. If the temperature remains much below 60 C., the color fails to develop or appears very faint.

The writer is aware that by the above method the differentiation between bile salts and cholesterin is the most uncertain point of the process, since it depends on the difference in diffusibility of the two substances through collodion sacs. It is quite certain that bile salts dialyze very rapidly from specimens of bile obtained at operation, or from fistulae after operation; but cholesterin has not been found to dialyze.

Furthermore, when a dialysate from a plasma is evaporated to dryness and extracted with ether or chloroform, the substance in this case which gives the positive Pettenkofer is left behind. Also, bile salts have been recovered from several plasmas by the Hoppe-Seyler method, and by dialysis as well, where cholesterin, as estimated by the method of Weston and Kent, was not increased. But the writer has not acquired proficiency in this cholesterin method, and draws no definite conclusions as yet on this particular point.

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8. Weston and Kent: Jour. Med. Research, 1912, **26**, 531.

The spectrum of a Pettenkofer test for bile salts, obtained from human gallbladder bile and fistula bile by dialysis and by the Hoppe-Seyler method, shows a wide absorption band in the blue when the test is first made and is of a cherry red color. Later, as the cherry turns to purple, the band in the blue fades and a smaller band develops in the orange (near *D*, between *D* and *C*). In a strong reaction both may be visible at once, but in weaker reactions the band in the blue usually fades before the other appears. This same spectrum is given by bile salts dialyzed from the plasma, as well as by those precipitated by the Hoppe-Seyler method. Numerous plasmas dialyze salts in water that give the Pettenkofer test; that is, the cherry red or pink color turning to purple, but not in concentration sufficient to allow the positive identification of the absorption bands. So we have been obliged to accept as positive in many instances reactions that give the colors, but not the spectra.

It is not claimed in this report that every specimen which gave a positive Pettenkofer test was proved absolutely to contain bile salts. But it may be said, further, that the method stated gives positive reactions in many cases of jaundice in which bile salts appear in the urine and gives negative tests in those same cases after the bile salts have disappeared from the urine. It also seldom gives positive tests in normal plasma.

The Pettenkofer reaction was not found to be adaptable to quantitative measurements either colorimetrically or spectroscopically, but there is a distinct variability in the intensity of the color reaction as well as in the definition of the absorption bands. This is sufficiently clear, that these tests can be classified as plus, double plus and triple plus.

By these methods the writer has examined the plasma of twenty patients in whom a diagnosis of pernicious anemia had been made. That is, he has tried Gmelin's reaction; measured the staining of the plasma; tested for urobilin; dialyzed and examined the dialysate for bilirubin and tested it for bile salts with the Pettenkofer and spectroscopic tests. The results of this investigation are summarized in Table 1. In this table also are certain other observations that are immediately concerned, namely, the blood findings relative to the anemia, the skin jaundice, the biliary elements in the urine, and the urobilin in the stool. On the urine the Hammersten-Salkowski test was used for the bile pigments, Hay's test for the bile salts, and Ehrlich's aldehyde test for urobilin. For the stool Wilbur and Addis' method of measuring the total daily urobilin excretion was used. And to correlate with the clinical side the table is made to include an index of the two most significant symptoms in the cases studied, that is, the loss of strength and the nerve disturbances. In the latter the determination of ataxias

is made by the plan demonstrated by Hoover[9] in 1914; acro-ataxia being an ataxia of the fingers and toes; proximo-ataxia, an ataxia of the iliofemoral or of the thoracicohumeral group of muscles.

RESULTS

Bilirubin: In brief, the table shows that out of the twenty cases, in sixteen there was found bilirubin in amounts sufficient to give a positive Gmelin test. In two of the remaining four the plasma was visibly jaundiced, but not deeply enough to give a test. The bilirubin showed a great variability, the figures ranging from 10 to 150. Of these, five specimens may be considered as containing bile above the threshold of the kidney, the threshold being established as 60 by similar observations on a group of cases of obstructive jaundice. However, bilirubin was found in the urine of none, regardless of the amount in the blood. Of the eighteen cases showing staining of the plasma, nine were visibly jaundiced in the sclerae; of the remaining nine, only one gave a history of ever being yellow.

Pigment dialyzed through the collodion sacs in but three cases. In these it was noticed on concentrating the dialysates that an unusually large amount of protein had dialyzed. In only one instance did sufficient pigment dialyze to allow its identification as bilirubin.

The cases showing the highest concentration of bilirubin showed the most marked evidence of rapid blood destruction, namely, increased output of urobilin in stool and urine, loss of strength from anemia, also the highest color index.

Urobilin: This was not found in the plasma of any, although it occurred in the urine of all save one, this one being a case which showed absolutely no staining of the plasma.

Salts: A positive Pettenkofer test was found in sixteen out of twenty plasmas. Of the four which did not give a positive Pettenkofer test, there were two plasmas which contained a large amount of bilirubin and two which contained none at all. There was no direct correspondence between the presence of salts or the amount of salts and the amount of bilirubin in the plasma. One plasma, with a jaundice of 75, gave a doubtful Pettenkofer, while two others with absolutely no staining of the plasma gave a strong Pettenkofer, and bile salts were found in the urine of none of the cases. There was no relation between the amount of bile salts and the blood destruction or the color index.

It was observed, however, that the cases giving the strongest Pettenkofer, hence highest concentration of salts, showed the most marked nerve lesions; in fact in three such cases the nerve lesions caused all the symptoms of the disease.

9. Hoover: Am. Jour. Med. Sc., 1915, **150**, 651.

By these same methods the writer has examined the plasma of a series of patients selected on account of suspected liver disease, infections, anemias, etc., as well as many normals. Table 2 summarizes ten of these cases, nine being secondary anemias from various causes, and one being a case of Banti's disease. Of the nine secondary anemia

TABLE 1.—SUMMARY OF FINDINGS—

Name	Age, Years	Red Blood Cells	Hemoglobin, Tallquist, %	Color Index	Jaundice	History of Jaundice	Blood		
							Jaundice of Plasma	Gmelin's Test	Urobilin
L. H.	60	1,082,000	30	1.5	+	+	20	+	...
*		3,500,000	70	1	+	+	15	0	...
M. C.	52	1,200,000	40	1.7	+	+	60	+	0
B. S.	62	3,200,000	70	1.05	+	+	...	+	...
†		3,200,000	70	1.05	+	+	15	0	...
‡	64	3,400,000	70	1	0	+	15	0	0
S. H	57	2,277,000	60	1.3	0	0	20	+	...
U. A.	52	2,312,000	70	1.5	0	0	0	0	...
F. C.	64	1,400,000	40	1.4	+	+	150	+	0
M. B.	48	3,824,000	80	1.05	0	0	15	0	...
L. C.	42	3,400,000	70	1	0	0	75	+	0
E. B.	39	1,206,000	70	2.9	0	0	50	+	0
J. W.	56	1,168,000	40	1.7	+	0	70	+	0
§		1,944,000	65	1.7	+	+	20	0	0
L. Y.	45	3,724,000	75	1	0	0	50	+	0
C. A.	60	2,256,000	75	1.7	+	0	50	+	...
#		1,200,000	45	2	+	+	25	+	0
H. K.	47	3,870,000	85	1.1	+	0	0	0	0
G. L.	42	574,000	40	1.8	+	+	70	+	0
F. F.	43	1,200,000	40	2	0	0	40	+	0
J. L.	56	3,122,000	80	1.3	0	0	25	+	0
M. H.	27	960,000	30	1.5	0	0	35	+	0
V. B.	39	2,626,000	65	1.5	0	0	25	+	0
B. P.	67	1,848,999	50	1.4	0	0	16	0	0
C. Z.	39	1,024,000	45	2.1	0	+	25	+	0

* L. H. three months later.
† B. S. three months later.
‡ B. S. one year later.
§ J. W. two months later.
# C. A. nine months later.

cases, none showed any staining of the plasma, although two of the patients were described by the ward physician as appearing jaundiced at the time the specimens were taken. Two of the secondary anemia cases had bile salts in the plasma. The patient with Banti's disease was jaundiced and had both bile pigments and salts in the plasma, but neither in the urine.

In a group of about forty cases, selected on account of liver disease, principally alcoholic and infectious cirrhosis, disease of the bile ducts, general infections with jaundice, and jaundice after transfusion, bile pigment and bile salts were found in the plasma in varying amounts, and in varying relations to each other and to choluria. The interpre-

—IN PERNICIOUS ANEMIAS

Dialysis		Urine			Stool Urobilin	Loss of Strength	Neurologic Symptoms			
Bile Pigment	Bile Salts	Bile Pigment	Bile Salts	Urobilin			Vibration Test	Acroparasthesias	Acro-Ataxia	Proximo-Ataxia
0	0	0	0	+	Increased.....	Marked.....	0	+	+	0
0	+	0	0	0	.....	Less marked..	0	0	0	0
0	+	0	0	+	Increased.....	Very great....	+	+	0	0
0	+	0	0	+	.....	Marked.....	+	+	+	0
0	+	0	0	0	.....	Marked.....	+	+	+	0
0	++	0	0	0	.....	Marked.....	+	+	+	0
0	+	0	0	+	Increased.....	Marked.....	+	+	+	+
0	++	0	0	+	.....	Slight.....	+	+	+	+
?	+	0	0	+	Much increased..	Great.....	0	0	+	+
0	0	0	0	+	Slight increase...	None.....	+	+	0	0
0	?	0	0	+	.....	None.....	+	+	+	0
0	?	0	0	+	Increased.....	Very great....	0	+	0	0
0	+	0	0	+	Much increased..	Very great....	+	+	+	0
0	+	0	0	0	Increased.....	Slight.....	+	+	+	0
0	+	0	0	+	.....	Slight.....	0	0	0	0
0	+	0	0	+	.....	Slight.....	+	+	+	+
0	+	0	0	0	.....	Slight.....	+	+	+	+
0	++	0	0	0	.....	Very great....	+	+	+	+
0	+	0	0	+	Increased.....	Marked.....	+	+	+	0
+	+	0	0	+	.....	Great.....	0	+	+	0
0	+	0	0	+	Increased.....	Slight.....	+	+	+	0
+	+	0	0	+	Increased.....	Very great....	0	0	0	0
+	+	0	0	+	Increased.....	Slight.....	0	0	0	0
0	0	0	0	+	.....	Marked.....	+	+	+	+
0	+	0	0	+	Increased.....	Marked.....	+	+	+	0

tation of the blood jaundice in the various conditions affords interesting speculation, but much remains to be done before any conclusions are justified. A few main points are clear, namely, that jaundice of the sclera or skin is of course always associated with jaundice of the plasma, but the converse is not true. We have frequently been surprised by obtaining a distinctly jaundiced plasma from a patient who had no suggestion of jaundice of the skin. Also, the presence of bile

salts in the blood is associated with some disease of the liver, which is in accord with the idea most commonly held, that bile salts are formed only in the liver.

On the basis of experience with jaundice of the plasma in the various diseases of the liver, and the frequent appearance of bile salts in the blood in pernicious anemia, one interpretation of our findings in the cases tabulated can be ventured, namely, that the jaundice is hepatic in origin. From the fact that bile pigment in all these cases was not found in the urine, and, furthermore, usually does not dialyze through collodion membranes into water, it is assumed that the pigment is fixed to the plasma, perhaps chemically. This fixation does not seem to depend on the intensity or duration of the jaundice. Why the bile salts are diffusible from the plasma through collodion membranes and do not appear in the urine we have no theory.

TABLE 2.—SUMMARY OF FINDINGS IN BLOOD—

Name	Age, Years	Diagnosis	Red Blood Cells	Hemoglobin, per Cent.	Color Index
A. B.	32	Hemorrhoids	2,144,000	20	0.47
J. S.	18	Bismuth poisoning	1,500,000	30	1
P. G.	30	Hemangioma of spleen	3,144,000	40	0.6
A. C.	32	Banti's disease	5,100,000	85	0.85
J. S.	28	Lead poisoning	4,640,000	70	0.7
L. Z.	34	Incomplete abortion	3,064,000	45	0.7
J. L.	25	Acute nephritis	2,888,000	50	0.9
W. H.	68	Carcinoma of stomach	4,010,000	70	0.9
H. S.	50	Chronic plumbism	3,680,000	70	0.9
L. C.	28	Hemorrhoids	2,500,000	30	0.6

What diagnostic value may be attached to the findings of bile pigment and bile salts in the plasma remains to be seen, but it is quite apparent that jaundice can be estimated with more certainty from observations of the plasma than from observations of the skin and sclerae. In the anemias, especially, a faint degree of jaundice, such as occurs in pernicious anemia, is frequently a matter of uncertainty, for the pallor exaggerates the normal pigmentation of the skin and the so-called lemon yellow skin is often a much disputed symptom. The large amount of bilirubin in the plasma in some cases increased the difficulties met in obtaining estimations of hemoglobin by the Tallqvist or Sahli method, for the adventitious yellow color prevents an accurate reading and tends to make the results too high. The diagnostic value of the presence of bile salts in the blood also is not very clear, but we

are inclined to accept such a finding as direct evidence of pernicious anemia in those cases in which anemia is a minor feature, and neurological signs and symptoms are the main features of the disease.

In the diagnosis of pernicious anemia we have relied principally on finding a marked anemia with a high color index associated in early cases, with parasthesias of the extremities, and other evidence of degeneration of peripheral nerves, and with degeneration of the posterior columns of the spinal cord in more advanced cases. In explanation of so large a series of pernicious anemia patients in twelve months in a clinic, such as is maintained at Lakeside Hospital, we can explain that some of them came by selection from a large private consulting practice.

As a method of prognosis, the measure of the jaundice of the plasma may be of considerable help, for it was found in three cases

—AND URINE IN SECONDARY ANEMIAS

Jaundice	History of Jaundice	Blood			Dialysis		Urine		
		Jaundice of Plasma	Gmelin's Test	Urobilin	Bile Pigment	Bile Salts	Bile Pigment	Bile Salts	Urobilin
+	+	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	+	15	0	0	0	0	0	0	0
+	+	40	+	0	0	+	0	0	+
0	0	0	0	0	0	+	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	10	0	0	0	0	0	0	0
0	0	20	0	0	0	0	0	0	0
0	0	15	0	0	0	+	0	0	0
0	0	0	0	0	0	0	0	0	0

that were observed, after a few months' interval, that the jaundice had diminished with the general improvement.

It is also seen that the patients kept on the ward and treated show a decrease in blood jaundice as they get relief from symptoms, the decrease usually being proportional to the increase in red cells.

SUMMARY

Bile pigment is frequently found in the blood in pernicious anemia, and is the cause of the jaundice. The bile pigment is in some way fixed to the plasma and therefore does not appear in the urine. The presence of bile pigment in the blood is not always betrayed by jaundice. Bile salts are frequently found in the blood, alone or associated with bile pigment. The jaundice in pernicious anemia is hepatic in origin.

REFRACTOMETRIC STUDIES OF SERUM PROTEINS IN NEPHRITIS, CARDIAC DECOMPENSATION, DIABETES, ANEMIA, AND OTHER CHRONIC DISEASES *

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In previous articles[1] we have reviewed the literature on serum proteins in health and disease, and have presented new values for serum proteins, obtained by Robertson's microrefractometric method, from normal adults and from those suffering with syphilis, pneumonia and other infections. The relation of globulin to the Wassermann reaction as well as the increase of serum proteins due to stasis have also been studied.

Former estimations of serum proteins in chronic diseases, which are referred to in a previous article,[2] show in a general way an increase of serum globulin with a decrease of total protein, the latter being most pronounced in hydremia. These results, though, are not uniform, owing to the use of varied technic and methods. We considered it necessary, therefore, to estimate the serum proteins in chronic diseases by the recent microrefractometric method of Robertson[3] which we have shown to be dependable and by which normal values have been already established.

NEPHRITIS

The refractometer has been used for many years to estimate total serum proteins in nephritis, but it has never been used to quantitate the serum albumin and globulin. Strauss[4] used the refractometer to study nephritic serums and thought his results offered a guide to treatment, since they showed the amount of edema present. He claimed,[5] moreover, that cardiac edema never produced such low dilution of serum as nephritic edema, with which opinion Widal,

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1. Rowe, A. H.: THE ARCHIVES INT. MED., 1916, **18,** 455; Jour. Lab. and Clin. Med., 1916, **1,** 439. Tranter and Rowe: Jour. Am. Med. Assn., 1915, **65,** 1433.
2. Rowe, A. H.: Footnote 1, first reference.
3. Robertson, T. B.: Jour. Biol. Chem., 1915, **22,** 233.
4. Strauss: Die Chronischen Nierentzündungen und ihre Einwirkung auf die Blut flussigkeit, 1902; Therap. der Gegenw., 1903, **44,** 433; Deutsch. med. Wchnschr., 1905, **31,** 83.
5. Strauss: Ztschr. f. klin. Med., 1906, **60,** 501.

Bernard and Vaucher[6] agreed, while Reiss,[7] in a recent article, disagreed. We, on our part, have not found as low total proteins in cardiac edema as in nephritic edema. Brandenstein[8] and Georgopulos,[9] pupils of Strauss, continued the study of serums of edematous patients, showing, among other things, normal values in compensated cardiac disease and in chronic nephritis. Reiss also found little variation from normal in total proteins in chronic nephritis. He obtained marked dilution of blood serum in acute nephritis associated with edema and showed that this dilution occurred before and lasted after the subcutaneous edema was visible. The effect of salt on body weight and serum concentration was studied. Chiray[10] also studied by the use of the refractometer the serum dilution which occurs in cardiac and nephritic cases.

In grouping the cases of nephritis in the accompanying tables, the effect of edema and of uremia has been shown. No characteristic values for serum proteins which might aid in the clinical diagnosis of the type of kidney lesion were found.

Two cases of acute nephritis were included in Table 4 of a previous article.[2] In Case 5 of this table circumscribed edema was present, but not enough general edema to depress the total proteins below normal. In Case 6, on the contrary, generalized edema was present and the total proteins were markedly lowered. Both cases showed definite increase in the percentage of globulin and in the nonproteins.

The effect of chronic nephritis and edema on the serum proteins is shown in Table 1 of this article. The most marked variation from normal is the low total protein. This is due, in large part, to the presence of hydremia, though the toxic effect of retained waste products undoubtedly plays a rôle in reducing the available body proteins. Moreover the percentage of globulin and the nonproteins are above normal. Charts 1 and 2 show the striking way in which the total proteins and albumin rise and the nonproteins fall with the decrease of body weight due to the loss of edema. The separation of the albumin and globulin curves with recovery from the severe stage of illness is very pronounced and is well shown in both charts, especially in Chart 2, in which at the first examination the albumin and globulin were equal in amount and in ten days the ratio between the two became normal, that is, 26 per cent.

6. Widal, Bernard and Vaucher: Semaine méd., 1911, **31,** 49.
7. Reiss: Ergebn. d. inn. Med. u. Kinderh., 1913, **10,** 603; Zentralbl. f. Kinderh., 1909, **14,** 150; Jahrb. f. Kinderh., 1909, **70,** 311; München. med. Wchnschr., 1908, **55,** 1853.
8. Brandenstein: Zur Frage der Schädigung der Nierenkrankheiten durch Kochsalz, Senator Festschrift, 1904.
9. Georgopulos: Ztschr. f. klin. Med., 1906, **60,** 411.
10. Chiray: Presse méd., 1908, **16,** 29.

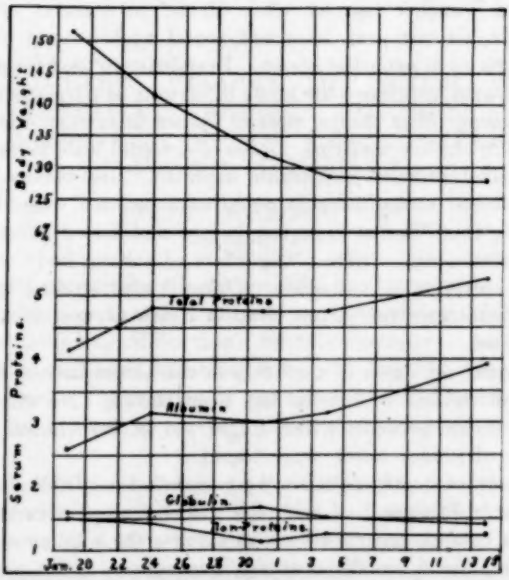

Chart 1.—Graphic results of Case 3 (Table 1). The low levels of serum albumin and total serum proteins in severe nephritis with edema followed by a rise in these values with clinical improvement and loss of edema is shown. The separation of the albumin and globulin curves in this chart is a definite index of recovery, though the failure of the total proteins to reach a more normal value indicates a bad prognosis.

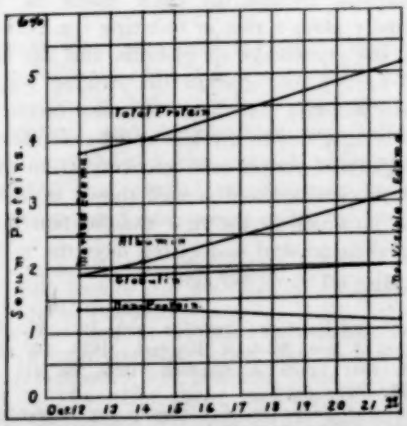

Chart 2.—Graphic results of Case 1 (Table 1). The comment of Chart 1 applies in this case. The separation of the albumin and globulin curves is most striking.

It is seen that the total protein at the last examinations made in Cases 1 and 3 were nearly 2 per cent. below the normal values, in spite of the complete absence of visible edema and a great loss of weight. This failure to return to the normal level is undoubtedly a manifestation of internal edema, which persists long after the subcutaneous edema is demonstrable, and also a manifestation of the chronic intoxication present in nephritis. That retained toxins can aid in breaking down serum proteins is certain. In this connection it is interesting to note that Castaigne and W. Chiray[11] have shown by

TABLE 1.—CHRONIC NEPHRITIS WITH EDEMA

Case No.	Age, Years	Date	Diagnosis, Data, etc.	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	34	10/12	Chronic nephritis with marked edema; slight rise of temperature and leukocytosis at entrance; Wassermann negative	1.9	1.9	3.8	1.4	50
		10/14	Nonprotein N = 56 mg. per 100 c.c. of blood; less edema	2.1	1.9	4	1.4	47.5
		10/22	No visible edema; walking around, feeling well	3.2	2	5.2	1.2	38
2	39	1/29	Chronic glomerular nephritis with marked edema; nonprotein N = 36 mg.; Wassermann wk. +; weight = 173 lbs.	3	1.6	4.6	1.2	35
		2/ 3	Less edema; no other improvement; weight = 162 lbs.	3	1.8	4.8	1.4	37.5
3	38	1/19	Chronic glomerular nephritis; marked edema; weight = 152 lbs.	2.6	1.6	4.2	1.5	38
		1/24	Edema disappearing with energetic eliminative treatment; weight = 144 lbs.	3.2	1.6	4.8	1.4	33
		1/31	Less edema; weight = 134 lbs.	3	1.7	4.7	1.2	36
		2/ 4	Edema practically gone; weight = 128 lbs.	3.2	1.5	4.7	1.3	32
		2/14	Edema gone; weight = 126 lbs.	3.9	1.4	5.3	1.3	26

the refractometer that subcutaneous injections of complex proteins cause slight diminutions in the serum proteins, which, they conclude, is due to the toxic effect of these injected substances. These authors, moreover, were able to produce cachexia and finally death by many repetitions of such toxic effects. Thus, in a case of nephritis whose serum proteins remain far below normal long after visible edema has disappeared we must assume a chronic toxicity. Reiss[7] learned to associate a bad prognosis with such cases. From the above considerations it is seen that the duration and extent of treatment of nephritics

11. Castaigne and Chiray, W.: Compt. rend. Soc. de biol., 1906, **60-61,** 220.

could be guided in a definite way by the rapidity with which the total proteins return to their normal limits and by the separation of the albumin and globulin curves from one another. If either of these phenomena fail, prognosis is bad and treatment should be persistently continued.

Table 2 shows the effect of chronic nephritis with uremia on the serum proteins. Strauss[4] and Reiss[7] thought that the refractometric investigation of serum proteins was useless because of the great increase in the nonproteins. Reiss was certain that the correction of 0.6 per cent. which Widal, Bernard and Vaucher allowed for the increase in waste products in uremic serums was too small. Because of the fact that constant values have been used for nonproteins in determining the serum concentration with the refractometer, former

TABLE 2.—CHRONIC NEPHRITIS WITH UREMIA

Case No.	Age, Years	Diagnosis, Data, etc.	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	29	Chronic glomerular nephritis; hypertrophy and dilatation of heart, slight edema	3.4	2.4	5.8	1.7	41
2	45	Chronic glomerular nephritis, 11/15/16..	4.8	2.3	7.1	1.4	32
		In uremic coma for 18 hrs., 9/22/16....	6	1.9	7.9	1.55	24
3	22	Chronic glomerular nephritis; 160 mg. nonprotein N per 100 c.c. blood	3.9	2.2	6.1	2	36
4	45	Subacute nephritis; 86 mg. nonprotein N per 100 c.c. of blood; phenolsulphonephthalein renal function = 15 per cent.	3.3	2.6	5.9	1.6	44
5	26	Chronic glomerular nephritis, beginning uremia; death 4 days later; Wassermann sl. +	5.5	2.1	7.6	1.75	28

results are not satisfactory. Thus Robertson's method, employing, as it does, a separate determination for nonproteins in each case, makes possible for the first time accurate determinations by the refractometer of serum proteins in cases of uremia.

Our results show that in uremic patients, the percentage of globulin is usually increased above normal. In those two patients nearest death the percentage of globulin was normal, which finding we are unable to explain. In this series the total proteins are normal in one half of the cases and moderately subnormal in the other half. These normal values indicate a dehydration of the body tissues due, in large part, to the inability to take any fluid into the system. The nonproteins in these cases are very high, as would be expected.

Table 3 shows the effect of chronic nephritis alone on the serum proteins. The majority of these results present a decided increase in

the percentage of globulin over the normal, though three of the values are within normal limits. The total proteins vary from normal to slightly subnormal values, due probably to the varying degrees of toxicity and internal edema present. The nonproteins vary from normal values to high abnormal values, which shows the varying degree of kidney damage present in different cases of chronic nephritis, and emphasizes the importance of laboratory investigation of all nephritics. This importance of laboratory results is best shown in Case 6, in which the total proteins are slightly below normal, the nonproteins and percentage of globulin markedly above normal, all of which variations point toward a likely return of the former uremic condition and suggest the importance of treatment in spite of the apparent health of the patient.

TABLE 3.—CHRONIC NEPHRITIS WITHOUT UREMIA OR EDEMA

Case No.	Age, Years	Diagnosis, Data, etc.	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	45	Chronic nephritis; chronic myocarditis; arteriosclerosis	4.4	2.5	6.9	1.2	36
2	19	Chronic nephritis; duodenal ulcer.....	6.1	1.8	7.9	1.1	23
3	26	Chronic glomerular nephritis.....	3.6	2.5	6.1	1.2	40
4	17	Chronic nephritis	3.8	2.3	6.1	1.4	38
5	40	Chronic glomerular nephritis; hypertrophy and dilation of heart; blood N = 40 mg.; phenolsulphonephthalein renal function = 25%; fundi showed marked hemorrhages and exudates	5.2	2.5	7.7	1.4	37
6	22	Chronic nephritis; 4 years before had uremic coma and albuminuric retinitis	3.9	2.4	6.3	1.8	38
7	34	Chronic nephritis; hypertrophy and dilation of heart; secondary anemia	4.8	1.3	6.1	1.6	21

CARDIAC DECOMPENSATION

The refractometric determinations of total proteins as done by Böhme and Reiss,[7] Widal,[6] Chiray[10] and others in cases of cardiac decompensation have shown certain interesting facts. The "internal edema," which may be present in spite of the absence of visible external edema, occurs in this condition as well as in nephritis. The fact that serum dilution is usually less in edematous cardiac patients than in edematous nephritic patients was commented on under the subject of nephritis. We have found, as did Böhme and Reiss, cases of generalized cardiac edema in which the total proteins are normal in amount. Chiray[10] also noticed this and offered as an explanation venous stasis, caused primarily by heart insufficiency, but helped by

TABLE 4.—CARDIAC DECOMPENSATION

Case No.	Age, Years	Diagnosis with Data	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	63	Decompensation of heart; arteriosclerosis (general and renal); chronic passive congestion; edema in legs	4	1.9	5.9	1.4	34
2	43	Slight decompensation; mitral disease; auricular fibrillation; exophthalmic goiter? slight edema	4.7	2.6	7.3	1.3	36
3	38	Decompensation; auricular fibrillation; anasarca	4	2	6	1.3	33
4	20	Mitral stenosis; aortic and tricuspid insufficiency; pericardial effusion; 1/18/16, wt. = 130 lbs.	3.4	3.2	6.6	1.45	49
4	20	2/12/16, compensation regained; wt. = 128 lbs.	5	1.9	6.9	1.2	28
5	62	Myocardial insufficiency; arteriosclerosis; pulsus alternans; edema	4.5	2.1	6.6	1.3	32
6	34	Myocardial insufficiency; tricuspid insufficiency; chronic mitral and aortic disease; no edema	4.6	2.7	7.3	1.5	37
7	67	Myocardial insufficiency, with edema; auricular fibrillation; chronic nephritis	4.3	2.1	6.4	1.2	33
8	22	Aortic and mitral insufficiency, with stenosis; edema	4	2	6	1.3	33
9	45	Decompensated hypertrophied and dilated heart; edema; general arteriosclerosis	3.8	2.5	6.3	1.3	40

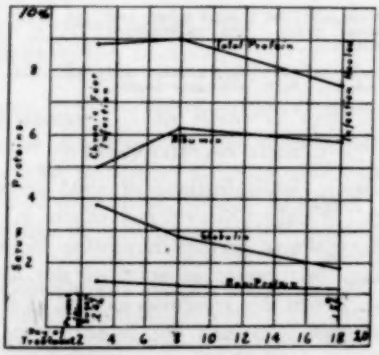

Chart 3.—Graphic results of Case 3 (Table 4). The high percentage of globulin due to a chronic foot infection in a diabetic with a fall of the globulin and separation of the albumin and globulin curves as the infection healed under the Allen starvation treatment is shown. The normal values for serum proteins obtained at the last examination are similar to those found in uncomplicated cases of diabetes.

kidney damage. This explanation has already been referred to in our recent article on venous stasis.[12] The percentage of globulin and the nonproteins are above normal in these cases of cardiac decompensation.

ARTERIOSCLEROSIS

The patients studied in this series had arteriosclerosis as their primary diagnosis and all had hypertension. Total proteins were normal except for moderate decrease in Cases 3 and 4. The percentage of globulin, except in Cases 2, 3, and 5, were within normal limits. The nonproteins were normal except in Cases 1 and 3. When the average results are computed they are found to be only moderately abnormal.

TABLE 5.—ARTERIOSCLEROSIS

Case No.	Age, Years	Diagnosis	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	46	Arteriosclerosis, including coronary sclerosis	5	2.6	7.6	1.5	34
2	76	Arteriosclerosis; cardiosclerosis; partial heart block; retention cystitis	4.2	2.3	6.5	1.3	35
3	70	Arteriosclerosis; emaciation; gastric ulcer?	4.2	1.9	6.1	1.4	31
4	62	Arteriosclerosis	4.2	2.7	6	1.3	45
5	50	Arteriosclerosis	4.8	3.1	7.9	1.1	39
6	53	Arteriosclerosis; hemiplegia	5.5	1.8	7.3	1.3	25
7	50	Arteriosclerosis; chronic nephritis.....	5.3	1.9	7.2	1.3	26
8	53	Arteriosclerosis; chronic nephritis; hypertrophied and dilated heart	5.1	2.5	7.6	1.2	33
9	56	Arteriosclerosis	5	1.8	6.8	1.3	26

DIABETES

Strubell,[13] Grober,[14] and Wagner[15] first used the refractometer to quantitate sugar in watery solutions and in diabetic urine. The latter application was not successful because of the many varying urinary constituents. Reiss[16] investigated serum proteins with the refractometer in later years, studying especially the relation of the serum concentration to the body weight and water balance in diabetes. He reviewed the literature on the blood concentration in diabetes. His conclusions

12. Rowe, A. H.: Footnote 1, second reference.

13. Strubell: Verhandl. d. Cong. f. inn. Med., 1900, **18,** 417; Deutsch. Arch. f. klin. med., 1901, **69,** 521; München. med. Wchnschr., 1902, **49,** 616.

14. Grober: Centralbl. f. inn. Med., 1900, **21,** 201.

15. Wagner: Ueber quantitative Bestimmungen wässer zur Lösungen mit den Zeisschen Entauchrefractometer, Dissertation, Jena, 1903.

16. Reiss: Deutsch. Arch. f. klin. Med., 1909, **99,** 419; see also Footnote 7.

were that changes in weight in diabetes are due to anomalies in the water and salt excretion caused by an accompanying nephritis.

The results of our series of examinations in diabetics are shown in Table 6. With the exception of Case 3, the percentages of globulin are all normal. The results of Case 3 are shown in Chart 3. The first examination was made when there was an active chronic infection in the right foot. This accounts, undoubtedly, for the high percentage of globulin present, since as the condition improved with Allen treat-

TABLE 6.—DIABETES

Case No.	Sugar in Urine, %	Blood Sugar, %	Comment	Albumin	Globulin	Total Protein	Non-protein	Per Cent. of Globulin
1	4	0.22	Before Allen treatment was started	6	2	8	1.3	25
2	0.8	0.24	Tests made during period of lowered tolerance	6.2	1.9	8.1	1.3	23
3	0	0.24	Third day of starvation, 39.8 vol. per cent. CO_2* bound by 100 c.c. of blood plasma; chronic infection in foot	5	3.8	8.8	1.4	43
	0	....	Eighth days of treatment, infection nearly well; low caloric intake	6.2	2.8	9	1.3	31
	0	0.15	Eighteenth day of Allen treatment, infection well; moderate caloric intake	5.8	1.8	7.6	1.2	24
4	0	....	Low caloric intake.....	5.7	1.3	7	1.3	19
5	0	....	Low caloric intake.....	5.5	1.7	7.2	1.2	24
6	0	....	Moderate caloric intake.....	6	1.9	7.9	1.2	24
7	2	0.36	Tuberculosis of the kidney with nephritis prevented a strict Allen treatment	5.6	1.8	7.4	1.4	24
8	0	....	Moderate caloric intake.....	5.7	1.5	7.2	1.3	21
9	0	....	Low caloric intake, 56.7 vol. per cent. of CO_2 bound by 100 c.c. of plasma	4.6	1.7	6.3	1.4	27
10	0	....	Starvation period; moderate degree of acidosis; 40.9 vol. per cent. of CO_2 bound by 100 c.c. blood plasma	4.2	1.5	5.7	1.2	26

* The volume percentage of carbon dioxid was determined by Van Slyke's method.

ment and the foot finally healed, the globulin became normal. The improvement in this case is indicated, as in Charts 1 and 2, by a separation of the albumin and globulin curves. That the high percentage of globulin was not due to a high blood sugar content is shown by the fact that Cases 1, 2, and 7, which had as large amounts of blood sugar as did Case 3, had perfectly normal amounts of globulin. All examinations, except in Case 1, were made on patients receiving the Allen starvation treatment under the direction of Dr. Orville

Rogers. That the Allen treatment was not responsible for the normal amounts of globulin obtained in all cases except Case 3 is shown by the normal results obtained in Case 1.

The average value for nonproteins in this series is slightly higher than normal. It is interesting to notice that as improvement occurred in Case 3, the nonproteins decreased to normal limits. Cases 9 and 10 had low total proteins, which probably was a sign that hydremia was present as well as a diminution of the available body proteins due to the chronicity of the disease and the long duration of low caloric intakes. That hydremia was in part a cause for these low values is likely when we consider the frequency of edema during the starvation treatment, especially when salty broths are freely given.

TABLE 7.—ANEMIAS

Case No.	Diagnosis	Blood Picture			Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
		Red Cells	White Cells	Hemoglobin, %					
1	Pernicious anemia...	1,336,000	3,000	45	4.5	1.3	5.8	1.2	22
2	Pernicious anemia...	1,200,000	3,700	30	4.4	1.4	5.8	1.3	24
3	Pernicious anemia...	1,152,000	8,800	30	5	1.6	6.6	1.3	24
4	Secondary anemia—etiology?	3,896,000	7,300	35	4.8	1.5	6.3	1.3	23
5	Secondary anemia; probable malignancy	2,888,000	38,000	60	4.4	1.7	6.1	1.3	28
6	Banti's disease.....	4,800,000	10,100	75	5	1.6	6.6	1.25	24
7	Malignant endocarditis	4,240,000	10,200	75	2.9	2.7	5.6	1.35	48
8	Parenchymatous nephritis; marked edema	?	6,800	95	2.6	1.6	4.2	1.5	38
9	Chronic nephritis; marked edema	4,600,000	8,800	80	1.9	1.9	3.8	1.4	50

ANEMIAS

Reiss[7] reports the refractometric determinations of total proteins of serums in two cases of pernicious anemia, these results varying between 6.4 and 5.1 per cent., with typical low blood pictures. Martius[17] obtained similar results and commented on the fact that the serums were not as dilute as he expected they would be from the examination of the whole blood. The literature on pernicious anemia, reviewed in the preceding paper, showed in a general way this same condition, as well as no increase in the percentage of globulin. The three cases of our series yielded results which agree with those in the

17. Martius: Folia haemat., 1906, **3**, 138.

literature. The serum proteins in secondary anemias due to cancer, Banti's disease, malignant endocarditis, and nephritis with edema are tabulated and are especially interesting in showing the remarkably high amount of total protein present in pernicious anemia as compared with the results in secondary anemias. A priori, one would assume that the lowest values for serum proteins would occur in pernicious anemia, in which one obtains the lowest number of red cells and very low percentages of hemoglobin. On the contrary, the lowest results occur where the red count and hemoglobin are much higher than in pernicious anemia.[19] In the last three cases of this series the percentage of globulin is high, as would be expected from the etiologic factors at work in the production of these anemias.

TABLE 8.—MISCELLANEOUS CHRONIC DISEASES

Case No.	Diagnosis, etc.	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	Hyperthyroidism	5.4	2	7.4	1.2	27
2	Hyperthyroidism	5.7	1.1	6.8	1.2	16
3	Goiter (colloid)	4.6	1.8	6.4	1.3	28
4	Hemophilia; R. C., 5,055,000; hemoglobin, 80%; bleeding time 2-2½ min.; clotting time 55 min.	5.9	2.5	8.4	1.3	30
5	Myeloma of spinal cord; Bence-Jones bodies in urine	4.8	2	6.8	1.5	29
6	Chronic bronchitis	5.7	2	7.7	1.3	26
7	Bronchial asthma	5.7	2	7.7	1.4	26
8	Pellagra	5.6	1.9	7.5	1.3	25
9	Obesity; Wassermann +++	6	1.6	7.6	1.2	21
10	Lead poisoning	5.5	1.8	7.3	1.1	25
11	Dyspepsia; constipation	5.4	2.1	7.5	1.2	28
12	Indigestion; gastric ptosis	5.3	1.7	7	1.1	24
13	Constipation	5.2	2	7.2	1	28
14	Duodenal ulcer	5	2.3	7.3	1.2	32
15	Cardiac neurosis	5.7	2	7.7	1.3	26
16	Neurasthenia	5	2.4	7.4	1.2	32

MISCELLANEOUS CHRONIC DISEASES

The cases of hyperthyroidism, goiter (colloid), hemophilia, chronic bronchitis, pellagra, obesity, lead poisoning, chronic gastro-intestinal disorders, and neurasthenia presented normal values for serum proteins. One case of bronchial asthma and a case of myeloma of the spinal cord which showed the Bence-Jones bodies in the urine had

18. It is interesting to know that Reiss (Arch. f. exper. Path. u. Pharmakol., 1914, **51**, 19) found normal values for total proteins in severe cases of leukemia.

moderate elevation of the nonproteins, with normal values for the serum proteins. How much this elevation of nonproteins means in the case of myeloma we are unable to say. The single case of obesity examined showed triple positive Wassermann, but this syphilitic condition failed to elevate the globulin. Our normal results in a case of hemophilia coincide with those recently obtained by Hurwitz and Lucas[19] by Robertson's microrefractometric method. It is interesting to include a note about the finding by refractometric examination of profound changes in serum proteins by Widal[20] during the attacks of paroxysmal hemoglobinuria. It was impossible for us to confirm this and to study any possible changes in serum albumin and globulin that may occur in this disease.

SUMMARY

1. Chronic nephritis with edema shows the lowest values for total serum proteins obtained in disease. The cause of these low values is probably chronic intoxication as well as hydremia. The percentage of globulin and the nonproteins are definitely increased above normal limits.

2. Serum proteins in chronic nephritis with uremia have been determined for the first time by an accurate refractometric method. The total proteins are either normal or slightly above normal, due probably to dehydration. The percentage of globulin is usually increased, but approaches normal as death draws near. Nonproteins are extremely high.

3. Chronic nephritis without edema or uremia produces a decided increase in the percentage of globulin, a moderate increase in the nonproteins and slight depression of the total proteins.

4. Cardiac decompensation with or without edema shows a decrease of total proteins, though not to the extent found in chronic nephritis with edema. The percentages of globulin and the nonproteins are moderately above normal.

5. In arteriosclerosis the average results show normal total proteins, while nonproteins and percentages of globulin are moderately increased.

6. Diabetes shows normal percentages of globulin except when a complicating infection is present, which causes a rise. A slight increase in the nonproteins was found. Total proteins were normal except for subnormal values in two severe cases.

7. In pernicious anemia the total proteins are not as low as would be expected from examination of the whole blood, being higher than

19. Hurwitz and Lucas: The Archives Int. Med., 1916, **17**, 543.

20. Widal, Abrami, Brissaud, Bernard and Joltrain: Arch. d. mal du Coeur, 1915, **8**, 430.

in nephritis with edema, in which the blood picture is much nearer normal than in the former disease. Nonproteins and percentages of globulin are normal in pernicious anemia and in secondary anemias due to cancer and Banti's disease, but increased in the cases of secondary anemia due to infection and nephritis.

8. Hyperthyroidism, goiter (colloid), hemophilia, chronic bronchitis, pellagra, obesity, lead poisoning, chronic gastro-intestinal disorders and neurasthenia gave normal results. One case of bronchial asthma and one case of myeloma with Bence-Jones bodies showed moderate elevation of the nonproteins with normal values for the serum proteins.

9. The accompanying charts show how recovery from the given illness produces a rise in total proteins, a separation of the albumin and globulin curves or a fall of the nonproteins. It is suggested that these phenomena might be used to help determine prognosis and the duration of treatment.

BACTERIOLOGIC STUDIES IN SUBACUTE STREPTOCOCCUS ENDOCARDITIS *

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It was the purpose of this study to search for distinctive features in the group of streptococci associated with subacute streptococcus endocarditis. The criteria adopted were cultural, biochemical and immunologic characteristics. Incidentally, the presence of antibodies in the blood of patients suffering from the disease and the immediate effect of blood transfusions on the bacteriemia were investigated.

The recognition of a distinct form of endocarditis, called by some writers subacute bacterial, by others endocarditis lenta and chronic infectious endocarditis, has been of comparatively recent date and of slow evolution. Although of only moderately common occurrence, the disease is extremely important, since all of the cases studied, except one,[1] have been fatal.

Virchow in 1855 first suggested an infectious origin for the endocarditis associated with uterine infections, and attention was thereafter directed to the finding of bacteria in the vegetations in both ulcerative and verrucous endocarditis. Heiberg[2] described a case of acute endocarditis which had been reported by Winge in 1869 in which micrococci were found in the vegetations and added another case of acute ulcerative endocarditis in which small micrococci were present in the vegetations.

For a short period it was thought that bacteria were present in all forms of endocarditis. Thus, Klebs[3] felt that he could separate rheumatic from septic cases by the character of the cocci found in microscopic sections. Köster[4] thought that verrucous endocarditis was a chronic and healed stage of ulcerative endocarditis and hence originally of bacterial origin. It may be noted that this idea of healed bacteria-free endocarditis has been supported with new evidence in recent years by Libman,[5] but with the distinction that the healed cases are supposed to have been of the subacute bacterial type.

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* From the Medical Clinic, Presbyterian Hospital, Columbia University, New York.

1. Hemsted, H.: Lancet, London, 1913, **1,** 10.
2. Heiberg, H.: Virchows Arch. f. path. Anat., 1872, **56,** 407.
3. Klebs: Arch. f. exper. Path. u. Pharmakol., 1878, **9,** 52, cited by Wyssokowitch (Footnote 8).
4. Köster, K.: Virchows Arch. f. path. Anat., 1878, **72,** 257.
5. Libman, E.: Am. Jour. Med. Sc., 1913, **146,** 525.

Later investigators failed to demonstrate bacteria in the lesions of verrucous endocarditis. Hamburger[6] examined ten such cases with negative results and Weichselbaum[7] questioned whether verrucous endocarditis was due to microbic invasion. This view received further support from Wyssokowitch[8] and Orth.[9]

Additional interest was lent to the discussion by the experimental production of acute endocarditis by previously puncturing the aortic leaflets according to a method devised by Rosenbach.[10] Wyssokowitch[8] was able to infect the injured valve by injecting pyogenic streptococci. Ribbert[11] produced acute endocarditis merely by injecting a suspension of staphylococci and fine particles of potato intravenously. Many reports of the production of endocarditis by injecting cultures of streptococci have been subsequently published.

As the facts then stood, there were two general types of endocarditis, namely, those associated with bacteria and those in which no direct connection with bacteria could be proved. The introduction of blood culture methods was responsible for separating the bacterial group into acute and chronic cases. Of the early reports, that of Kühnau[12] is noteworthy, in that sixty-six cases of rheumatism, many of which were accompanied by endocarditis, gave negative cultures, the finding of sarcinae, staphylococci and hay bacilli being discounted.

It soon became evident that among those cases of endocarditis which gave definitely positive blood cultures a certain group could be distinguished because of their subacute or chronic character. These cases most frequently yielded streptococci from blood cultures. This was noted by Harbitz.[13] Later Horder[14] very accurately described cases of acute and chronic infectious endocarditis of which seventeen were subacute and chronic and yielded blood cultures of "saprophytic streptococci." Osler at the same time pointed out the importance of the latter group. In the following year Schottmüller[15] individualized this type of endocarditis with the name "endocarditis lenta," a disease of slow, insidious onset, definite bacteriology and symptoms of injured heart valves with bacteriemia. The five cases which he described were caused by streptococci of the green-producing variety. In the

6. Hamburger: Ueber akute Endocarditis in ihrer Beziehung zu Bakterien, Berlin, 1879, cited by Wyssokowitch (Footnote 8).
7. Weichselbaum: Centralbl. f. Bakteriol., 1887, **2,** 209.
8. Wyssokowitch, W.: Virchows Arch. f. path. Anat., 1886, **103,** 301.
9. Orth, J.: Virchows Arch. f. path. Anat., 1886, **103,** 333.
10. Rosenbach, O.: Arch. f. exper. Path. u. Pharmakol., 1878, **9,** 1, cited by Wyssokowitch (Footnote 8).
11. Ribbert: Deutsch. med. Wchnschr., 1885, **11,** 717.
12. Kühnau, W.: Ztschr. f. Hyg. u. Infectionskrankh., 1897, **25,** 492.
13. Harbitz, F.: Deutsch. med. Wchnschr., 1899, **25,** 121.
14. Horder, T. J.: Quart. Jour. Med., 1908-1909, **2,** 289.
15. Schottmüller, H.: München. med. Wchnschr., 1910, **57,** 617.

same year Libman and Celler[16] described the same disease under the name of "subacute bacterial" endocarditis and found later[17] that while most of the streptococci produced green on blood agar plates, some were indifferent and some produced slight clearing. They also stated that the streptococci found were not very virulent.

The fact that positive blood cultures are occasionally obtained in rheumatic endocarditis should not confuse the conception of the disease under discussion, because bacteriemia in rheumatic endocarditis is rare, slight and transitory when it does occur, or may be present just before death. Subacute bacterial endocarditis, on the other hand, is a disease in which bacteriemia is a constant feature. This question is well discussed in a review by Simons.[18]

While the bacteria obtained are almost always streptococci, other organisms, such as influenza bacilli and gram-negative cocci have been described. The cases on which the present study was based were all cases of streptococcus infection. The characteristics of these streptococci have been studied by others, and such features as low virulence and their effect on blood agar have been described. Horder[14] studied the fermentation reactions of the streptococci from seventeen such cases from the standpoint of the grouping proposed by Andrewes and Horder[19] and found that nine belonged to the salivarius type and eight to the fecalis type. Major[20] studied the complement fixing antibodies in the serum of a patient and concluded that the reaction was not specific, since it was also obtained with an antigen made from *Streptococcus pyogenes*. Rosenow[21] claims that the bacteria usually isolated from these cases are "modified pneumococci," and tries to show that "the continuation of infection and death are largely due to a process of bacterial immunization against the antibodies of the host rather than to the virulence in the usual sense of the infecting bacteria."

CASE REPORTS

The twelve cases herein described were typical of the disease. A brief synopsis of each case follows:

CASE 1.—R. T., ironworker, aged 43, gave an indefinite history of rheumatism. He had "typhoid-pneumonia" twenty years previously. He had pains about the heart for twenty years. The onset of the present trouble was gradual, with weakness and chills. Later features were petechiae, subcutaneous nodules, arthritis in the small joints of hands and feet, left-side cardiac enlargement and signs of aortic and mitral insufficiency. The Wassermann reaction was negative.

16. Libman, E., and Celler, H. L.: Am. Jour. Med. Sc., 1910, **140,** 516.
17. Libman, E.: Am. Jour. Med. Sc., 1912, **144,** 313.
18. Simons, I.: Quart. Jour. Med., 1913-1914, **7,** 291.
19. Andrewes, F. W., and Horder, T. J.: Lancet, London, 1906, **2,** 775.
20. Major, R. H.: Bull. Johns Hopkins Hosp., 1912, **23,** 326.
21. Rosenow, E. C.: Jour. Infect. Dis., 1909, **6,** 245; ibid., 1910, **7,** 429.

The leukocytes varied from 10,000 to 15,000, the temperature from 99 to 100. Streptococcus *A* 4 was isolated repeatedly from blood cultures during life and from the blood and spleen postmortem. Six months before death there were sixteen colonies per cubic centimeter and a few days before death 165. The duration was thirteen months.

Case 2.—T. O., a carpenter, aged 34, gave a history of chorea, "malaria" and gonorrhea, but not of rheumatism. The onset of the present illness was gradual, with weakness, fever, chills and signs of aortic and mitral regurgitation. He was under observation only twelve days before death. The same streptococcus *O* was recovered in pure culture three times. The total duration was about two months.

Case 3.—C. S., a laundryman, aged 42, had sinus infection several months before admission. The onset of his present illness was gradual, with weakness. The features were purpuric eruption, necrotic area on the feet and lower legs and terminal hemiplegia. There were signs of marked aortic regurgitation. The leukocytes numbered from 25,000 to 35,000. The Wassermann reaction was negative. The temperature ranged from 99 to 103. Streptococcus *A* 30 was recovered repeatedly. There were forty-three colonies per cubic centimeter. The duration of the illness was about four months.

Case 4.—M. S., a married woman, aged 31, gave a history of "inflammatory rheumatism" at the age of 15 and again at 25. The onset of this illness was slow, with "muscular rheumatism." After seven weeks she began to have fever, joint pains and palpitation. She was in bed six weeks longer. She entered the hospital with signs of marked mitral insufficiency, tenderness in the small joints of the hand, leukocytosis of from 12,000 to 16,000, and temperature of from 101 to 104. Streptococcus *XK* was isolated three times during three weeks, at the end of which time the patient died of cerebral embolism. The total duration of the illness was about four months.

Case 5.—M. H., a single woman, aged 36, gave a history of scarlatina, diphtheria and frequent sore throat in childhood. She had acute articular rheumatism at 21, accompanied by "heart trouble." There had been dyspnea for the previous four years. The onset of this illness was gradual, with joint pains, chills, weakness and palpitation. The clinical features were petechiae, enlarged spleen and signs of aortic obstruction and regurgitation and mitral regurgitation. The Wassermann reaction was negative. The leukocytes varied from 6,000 to 15,000, and the temperature from 101 to 104. Streptococcus *B* 29 was isolated repeatedly during life, 175 colonies per cubic centimeter, and in pure culture from the aortic vegetations postmortem.

Case 6.—M. R., a junk dealer, aged 46, gave no history of previous infections of any kind. Rheumatism was denied. The onset of his illness was very insidious, with weakness, fever and occasional sweat. Examination revealed signs of aortic insufficiency. Later there were petechiae. The leukocytes varied from 12,000 to 15,000, the temperature from 100 to 102. The Wassermann reaction was negative. Streptococcus *"R"* was isolated four times over a period of two months. The patient died of cerebral embolism. The total duration of the illness was about three months.

Case 7.—F. M., a married woman, aged 22, had measles in childhood, chorea at 6, diphtheria at 12, and rheumatism at 12. She had had palpitation from the age of 7. She was said to have had "aortic regurgitation" for years. The onset of this illness occurred with headache, weakness and gastro-intestinal disturbance. The clinical features were pain in the left shoulder and signs of aortic and mitral insufficiency. The leukocytes numbered 7,000, and the temperature varied from 101 to 103. The Wassermann reaction was negative. Streptococcus *A* 148, 170 colonies per cubic centimeter, was recovered repeatedly during life and from the blood after death. The duration of the illness was about two and a half months.

CASE 8.—J. M., a boy, aged 18, gave a history of acute articular rheumatism at 12 and 16 years, accompanied by "heart trouble." The onset of his illness was gradual, with palpitation, precordial pain and joint pains. The clinical features were a tender joint of one of the small toes and signs of aortic and mitral insufficiency. The leukocytes numbered 18,000. The Wassermann reaction was negative. The temperature varied from 101 to 103. Streptococcus *B* 4 was isolated repeatedly, 570 colonies per cubic centimeter. The patient died after an illness of about five months.

CASE 9.—R. M. B., a physician, aged 34, gave a history of acute articular rheumatism at the age of 13. He was known to have had "aortic regurgitation" for ten years. The onset of this illness was gradual, with weakness, continuous fever and joint pains. The clinical features were signs of aortic insufficiency, enlarged spleen, arthritis of the small joints of the hand, petechiae and subcutaneous nodules. Streptococcus *MB* was isolated four times in pure culture, from four to six colonies per cubic centimeter, over a period of four months. The duration of his illness was seven months. Pure culture of streptococci were obtained postmortem from the heart's blood and from the spleen; and streptococci were present in the smears of aortic vegetation.

CASE 10.—P. S., a boy, aged 15, had scarlet fever followed by "swelling of the legs" at 4; joint pains and "heart trouble" at 6; attack of cardiac weakness with fever at 8; similar attack with pain in the feet, neck and abdomen with sterile blood culture at 11. The present trouble began with pain in feet, ankle and knee, with petechiae, subcutaneous nodules and progressive weakness. The clinical features were tenderness over the spleen, petechiae and signs of aortic and mitral insufficiency. The leukocytes numbered 14,000. The temperature ranged from 99 to 102. The Wassermann reaction was negative. Streptococcus *A* 26 was isolated frequently over a period of ten months, there being seventeen colonies per cubic centimeter. The duration of the illness was about eleven months.

CASE 11.—C. C., laundryman, aged 36, gave a history of occasional sore throat. There was a slow onset of the present illness, with continuous fatigue, occasional chill, loss of weight and cough. The clinical features were signs of marked aortic insufficiency. The temperature ranged from 98 to 102. The leukocytes numbered from 8,000 to 10,000. The Wassermann reaction was negative. Streptococcus *A* 56 was isolated repeatedly, from 165 to 200 colonies per cubic centimeter. The duration of the illness was about eight months.

CASE 12.—A. B., a married woman, aged 33, gave a history of scarlatina and diphtheria in childhood and "malaria" with "pneumonia" at 15. There was a vague history of rheumatism. The onset of the present illness was with sore throat, followed by chills and later, by joint pains and painful finger tips. The clinical features were petechiae, subcutaneous nodules, pain in the hips and signs of mitral insufficiency and stenosis. The leukocytes numbered 15,000. The Wassermann reaction was negative. The temperature ranged from 99 to 103. Streptococcus *A* 32 was isolated frequently during three months, from 500 to 1,200 colonies per cubic centimeter. The patient died about four months after the onset.

METHODS

1. Blood Cultures: From 30 to 40 c.c. of blood were withdrawn from a cubital vein by means of a McRae needle. The blood was planted directly in flasks of broth, from 5 to 10 c.c. of blood being used. Dextrose agar plates were poured (from 2 to 3 c.c. of blood each) and 5 c.c. were hemolyzed in 40 c.c. of sterile distilled water and the sediment, after centrifugalization, was planted in a deep tube of

melted ascitic dextrose agar and allowed to harden before incubating. For the purpose of noting the effect of transfusion on the number of colonies per cubic centimeter plates were poured with a definite amount of blood just before and at intervals after the transfusion.

2. For the purpose of demonstrating antibodies in the serums of patients or animals, agglutination reactions, "thread" reactions, and complement fixation reactions were done as follows:

a. Agglutination Reactions: Dilutions of serum were made in tubes containing 0.5 c.c. dextrose broth, so that the first tube contained 0.1 c.c. of serum, the second 0.05 c.c., the third 0.025 c.c., etc. To each tube 0.5 c.c. of dextrose broth culture was added. The mixtures were placed in the water bath at 37 C. for two hours and readings were made after the tubes had remained on ice over night.

b. "Thread" reactions were done by making dilutions of serum in tubes containing 1 c.c. dextrose broth so that the first tube contained 0.1 c.c. of serum; the second 0.01 c.c., the third 0.001 c.c., etc. The tubes were then inoculated with a drop of active dextrose broth culture and allowed to incubate for twelve hours. When the reaction was positive, the growth was restricted to an agglutinated pellicle in the bottom of the tube. Tubes containing normal serum were used as controls. The "thread reaction" was originally employed by Pfaundler[22] for testing the agglutinin content of antityphoid serums and has been further developed by Mandelbaum,[23] Gaehtgens and Kamm[24] and Dennemark.[25]

c. Complement Fixation Reactions: Two units of complement and antisheep hemolytic amboceptor were used with 0.5 c.c. of 5 per cent. saline suspension of sheep cells, 0.1 c.c. of patient's serum, 0.1 c.c. antigen and saline enough to make the final volume 2.5 c.c. The antigen was prepared as follows: The washed sediment of 125 c.c. of a twenty-four-hour broth culture was emulsified in 5 c.c. saline and precipitated by the addition of 5 c.c. absolute alcohol. After centrifugalization the supernatant fluid was discarded and the precipitate dried in vacuo, ground and weighed. Ten mg. were dissolved in 5 c.c. of 2 per cent. antiformin at 56 C. and neutralized with normal sulphuric acid, litmus paper being used as an indicator. The free chlorin was liberated by adding one or two drops of 5 per cent. sodium thiosulphate solution, the end result being tested with potassium iodid starch paper. The solution was then made up to 10 c.c. with carbolized saline so that 1 c.c. contained 1 mg. of dried precipitated bacteria.

22. Pfaundler, M.: Centralbl. f. Bakteriol., 1898, **23,** 131.
23. Mandelbaum, M.: München. med. Wchnschr., 1910, **57,** 178.
24. Gaehtgens, W., and Kamm, W.: München. med. Wchnschr., 1910, **57,** 1389.
25. Dennemark: Centralbl. f. Bakteriol., 1911, **58,** 354.

3. The cultural characteristics of the streptococci included (*a*) their appearance in broth, (*b*) their effect on serum dextrose agar, (*c*) their solubility in bile, (*d*) the lethal dose of a twenty-four-hour broth culture for white mice, and (*e*) their effect on red blood cells. The last effect was tested by planting cultures on blood agar plates and by mixing a twenty-four-hour broth culture with a 5 per cent. saline suspension of sheep red blood corpuscles according to the method recommended by Lyall.[26] (*f*) The fermentation reactions on litmus milk and on raffinose, inulin, salicin and mannite serum water mediums were studied. Each strain was started from a single colony.

4. For the purpose of attempting a classification of the streptococci on an immunologic basis, rabbits were inoculated with saline emulsions of killed streptococci at four-day intervals in doses equivalent to 10 c.c. broth culture. The injections were continued until the serum of an animal showed marked complement-binding capacity for the corresponding streptococcus antigen. Then the serum was tested against the other strains for cross fixation and cross agglutination.

RESULTS

1. Blood cultures: (*a*) As indicated in the case reports, each patient repeatedly yielded a positive blood culture. The colonies were always present in all the mediums; the streptococcus isolated was always in pure culture and identical with the organisms of each of the other cultures in the same case. The number of colonies varied from five to 1,200 per cubic centimeter. The bacteriemia did not fluctuate or change with rapidity and the same count might be expected from day to day. However, there seemed to be a gradual increase in the number of colonies per cubic centimeter as the disease advanced. The degree of bacteriemia did not seem to bear any direct relationship to the duration of the disease.

b. Effect of transfusion on the bacteriemia (Table 1): In four patients the effect of transfusion on the number of streptococci in the circulating blood was studied. Two different methods of transfusion were employed: (1) The Lindemann syringe method, (2) defibrination of the blood by wire or beads immediately on withdrawal from the donor, filtration through gauze and injection without the addition of saline. In the Lindemann method normal salt solution was injected to determine whether the needle was properly placed and at intervals during the transfusion when difficulty was experienced in securing blood from the donor. The syringes were also washed frequently with saline. The saline used in these transfusions had stood in the flasks several days after sterilization and the water from which the saline

26. Lyall, H. W.: Jour. Med. Research, 1914, **30**, 487.

was prepared was not freshly distilled. It was later shown that the severe reactions following transfusions could be largely eliminated by the use of freshly prepared saline. In Cases 1 and 10 the transfusions with accompanying febrile reactions were followed by complete sterilization of the blood for twenty-four hours. In Case 11 transfusion with defibrinated blood on three different occasions was unattended by febrile or other unpleasant reactions and the bacteriemia was not affected. In Case 12 the two facts illustrated by the three previous investigations was confirmed, but the additional evidence was brought forward that the injection of 300 c.c. of impure saline was followed by a severe febrile reaction. This resulted in as marked temporary reduction in the degree of bacteriemia as followed the injection of blood

TABLE 1.—EFFECT OF TRANSFUSIONS ON BACTERIEMIA

Case No.	Streptococcus	Transfusion with	Febrile Reaction	Colonies per Cubic Centimeter				
				Before	At Time of Reaction	24 Hours	48 Hours	72 Hours
1	A 4	Whole blood, Lindemann method	Severe	8	....	0	....	9
		Whole blood, Lindemann method	Severe	25	....	0	....	24
10	A 26	Whole blood, Lindemann method	Moderate	17	....	0	....	8
11	A 56	Defebrinated blood....	None	135	....	135		
		Defebrinated blood....	None	195	....	123		
		Defebrinated blood....	None	165	165			
12	A 22	Whole blood.....	Severe	1,200	800	15		
		Defebrinated blood....	None	1,200	....	920	....	1,500
		300 c.c. saline.....	Severe	750	40			

and saline. It seems evident, therefore, that the reduction in bacterial count is due to the reaction on the part of the patient and not to the introduction of complementary substances or antibodies in the donor's blood.

2. Immune Bodies in Patient's Serum (Table 2): The serum of each patient was tested with one or two of the immune reactions as shown in Table 2. The organisms *A* 56 and *A* 32 grew in an agglutinated mass so that the agglutination or thread reaction could not be applied to the serum in Cases 11 and 12.

It will be seen that all the serums contained antibodies against the homologous organisms. This is most markedly exemplified in the thread reaction, in which the tendency to grow in clumps in dilutions of immune serum was most striking.

TABLE 2.—IMMUNE REACTIONS WITH PATIENTS' SERUM AGAINST HOMOLOGOUS STREPTOCOCCI

Case No.	Streptococcus	Agglutination Reaction	Thread Reaction	Complement Fixation Reaction
1	A 4	.....	1-10 +++ 1-100 ++ 1-1000 +	
2	O	1-10 + 1-20 + 1-40 +		
3	X K	.....	1-10 ++ 1-100 ++ 1-1000 +	
4	A 20	1-10 ++ 1-20 ++ 1-40 +	1-10 +++ 1-100 ++ 1-1000 +	
5	B 29	.....	1-10 ++ 1-100 + 1-1000 +	0.1 c.c. ++ 0.05 c.c. — —
6	R	1-10 + 1-20 + 1-40 ±	1-10 + 1-100 + 1-1000 +	
7	A 148	1-10 + 1-20 + 1-140 +	1-10 ++ 1-100 ++ 1-1000 +	0.1 c.c. —
8	B 4	.....	1-10 +++ 1-100 ++ 1-1000 +	0.1 c.c. + 0.05 c.c. + 0.025 c.c. —
9	M B	.....	1-10 ++ 1-100 ++ 1-1000 —	
10	A 26	.....	1-10 +++ 1-100 ++ 1-1000 +	0.1 c.c. ++++ 0.05 c.c. ++++ 0.025 c.c. ++++

3. The cultural characteristics and virulence for white mice are shown in Table 3. It will be seen that not all the organisms can be termed viridans, since four were indifferent in their action on blood cells. The term "mitis" is appropriate, since lack of virulence is a characteristic of all the strains. It is noteworthy that the four indifferent strains grew with clear supernatant broth and showed short chains and a disposition to clump. Among the strains which grew with turbidity the sediment was more granular when first isolated than later, due possibly to the presence of agglutinating substances carried from the blood of the patient by the bacterial bodies through two or three generations.

4. Classification: This was considered from the standpoints of fermentation, agglutination and complement fixation reactions.

a. The fermentation reactions are given in Table 4, in which the organisms are arranged according to groups. All of the strains, except *A* 32, which was lost, were tested when first isolated and again after an interval of from three to eighteen months. Only one organ-

TABLE 3.—Cultural Characteristics

Case No.	Streptococcus	Growth in Plain Broth	Ascitic Dextrose Agar	Bile Solubility	Effect on Red Blood Cells	Lethal Dose for White Mice
1	A 4	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
2	O	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
3	X K	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
4	A 30	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
5	B 29	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
6	R	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
7	A 148	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
8	B 4	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
9	M B	Granular sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.
10	A 26	Soft white sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.
11	A 56	Flocculent sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.
12	A 32	Granular sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.

TABLE 4.—Fermentation Reactions *

Streptococcus	Time in Months Between 1st and 2d Test	Number of Generations between 1st and 2d Test	Milk	Raffinose	Inulin	Salicin	Mannite
A 4	18	10	+	—	—	—	—
O	15	4	+	—	—	—	—
X K	11	5	+	—	—	—	—
A 30	18	10	+	+	—	—	—
B 29	3	3	+	+	—	—	—
R	13	7	+	+	—	—	—
A 148	14	5	+	+	—	—	—
B 4	8	2	+	—	+	+	—
M B	8	3	+	—	—	+	—
A 26	15	10	+	—	—	+	+
A 56	15	8	+	+	+	—	+
A 32	..	..	±	—	—	—	—

* In this table + means acid and clot; ± means acid and no clot. The reactions given in the table are those given recently by strain A 148.

ism, *A* 148, changed; it fermented inulin and salicin when first isolated and twelve months later fermented only raffinose on three different trials. The later reading is indicated in the table. This change in fermentation reaction does not remove it from the salivarius group. According to this table, there are two members of the fecalis group, the remainder being members of the salivarius group.

b. Complement Fixations: A rabbit was immunized to each strain, except *A* 32, which was lost; an antigen, however, was made of some dried sediment of *A* 32 and tested against a few of the immune serums. Five out of the eleven rabbits' serums were tested before immunization and gave no fixation with any of the eleven antigens.

The relations of the streptococci are given in Table 5. It will be noted that *A* 26, *M B*, *A* 56 and *A* 32, which are indifferent in their action on blood cells, fall (as far as the limited tests on *A* 32 can be of value) more or less into a common group. Moreover, *A* 26 and *A* 56 are the mannite fermenters. The raffinose fermenters are fixed, variously, by serums of animals immunized by members of the other groups. There is no obvious relationship between the grouping made on the basis of fermentation reactions and that on the basis of complement fixation reactions. Viewed from the standpoint of complement fixation reactions alone, it will be seen that the strains arrange themselves in two general groups—*A* 26, *M B*, *A* 56, *A* 32; and *R*, *X K*, *A* 30, *B* 29 and *O;* with members *A* 148 and *B* 4 forming strong bonds between. Yet the members in the upper and lower portions of the chart can be said to be independent.

c. The serums of the same rabbits, of the same or nearly the same dates, were used in the agglutination reactions as were employed in the complement fixation reactions. It soon became apparent that agglutination was confined to a narrower zone than complement fixation and was more likely to be restricted to homologous serums and bacteria. It will be seen that the only discrepancy is in the case of streptococcus *R*, which is agglutinated by serum *B* 4, whereas it is fixed only by its homologous serum; and it will be further noticed that serum *R* agglutinates streptococcus *B* 4, but does not give fixation with *B* 4. Accordingly the boundary line established by the agglutination reaction is probably the more correct in this case.

COMMENT

A résumé of the various studies in classification shows that the organisms all fall definitely into the nonhemolytic streptococcus group. According to their action on red blood cells they fall into two subgroups, namely, green producers, so-called viridans, and indifferent strains, so-called saprophiticus. The last-named strains all gave a

TABLE 5.—Complement Fixation Reactions *

Rabbit Serums Immune to Streptococcus	A 4	A 26	M B	A 56	A 32	A 148	B 4	R	X K	A 30	B 29	O
A 4	+	—	—	—	0	—	—	—	—	—	—	—
A 26	+	+	+	—	—	—	—	—	—	—	—	—
M B	—	±	+	+	+	+	+	—	—	—	—	—
A 56	—	±	+	+	—	+	+	—	—	—	—	—
A 32	0	0	0	0	0	0	0	0	0	0	0	0
A 148	—	—	—	—	0	+	+	—	—	—	—	—
B 4	—	—	—	—	0	±	+	—	—	—	—	—
R	—	—	—	—	0	—	—	+	—	—	—	—
X K	—	—	—	—	0	+	+	+	+	—	—	—
A 30	—	—	—	—	0	±	±	—	—	±	±	—
B 29	—	—	—	—	0	—	—	+	—	+	+	±
O	—	—	—	—	0	—	—	+	—	+	+	+

* In this table + indicates reactions of practically the same strength with homologous and heterologous antigens; ± indicates that reactions were only weakly positive; — indicates negative reactions; and 0 indicates reactions not done.

TABLE 6.—Agglutination Reactions *

Rabbit Serums Immune to Streptococcus	A 4	A 26	M B	A 56	A 32	A 148	B 4	R	X K	A 30	B 29	O
A 4	+++ —	—	—	—	0	—	—	—	—	—	—	—
A 26	—	++ ++	—	—	0	—	—	—	—	—	—	—
M B	—	—	++ +	+	0	—	—	—	—	—	—	—
A 56	—	—	—	++ +	0	—	—	—	—	—	—	—
A 32	0	0	0	0	0	0	0	0	0	0	0	0
A 148	—	—	—	—	0	++	++	—	—	—	—	—
B 4	—	—	—	—	0	++	++ ++	++ +	—	—	—	—
R	—	—	—	—	0	—	++ +	++ +	—	—	—	—
X K	—	—	—	—	0	—	++	—	++	—	—	—
A 30	—	—	—	—	0	+++	++ +	++	—	+++	—	—
B 29	—	—	—	—	0	—	—	—	—	—	+++	—
O	—	—	—	—	0	—	—	—	—	—	—	+++

* In this table the plus signs indicate the intensity of reaction; — indicates negative reaction of some degree of agglutination, as given with normal rabbit serum; and 0 indicates reaction not done.

granular precipitate in broth and fell roughly into one group by cross fixation reactions. Cross agglutinations among members of this group, as well as among the other strains, are generally lacking, the only exceptions being noted in strains *A* 148 and *B* 4. On the other hand, cross complement fixation permits a rough classification into two larger groups, with the two strains *A* 148 and *B* 4 as connecting links. Strains *M B* and *A* 56 seem to be closely related by means of immune bodies, as do *B* 29 and *O*. Each of these three pairs is composed of members that have different fermentation reactions, indicating that immune and biochemic characteristics may be quite distinct. Furthermore, grouping according to fermentation reactions does not correspond to differences in effect on red blood cells.

In practically every way, the various organisms appear to be distinct from one another. This fact emphasizes the futility of a general or shot gun serum therapy or vaccination. If specific immune therapy is to be of avail, it must be induced by means of the homologous organism. The demonstration of immune bodies in the serum of patients seems to indicate that the patient is making an effort to combat the infection. It was thought that a third factor, such as complement, might be lacking in this disease and that transfusion of normal blood might supply this missing substance. That the hypothesis was not correct seems to be indicated by the failure to obtain any diminution of bacteriemia following transfusion unaccompanied by febrile reaction, and the fact that temporary diminution in bacteriemia occurred when febrile reactions were induced by either transfusion or injections of impure saline. These results are of striking interest in view of the recent ideas concerning nonspecific factors in the treatment of infections as expounded by Jobling and Petersen[27] and Miller and Lusk.[28] Our results show conclusively that the nonspecific febrile reaction was followed by a temporary increase in antibacterial power in the blood. The various factors concerned in the increase have not been determined.

CONCLUSIONS

1. Subacute streptococcus endocarditis is a disease of definite bacteriology, each case yielding a constant individual type of organism.

2. The streptococci belong to the saprophytic types according to Andrewes and Horder's classification and are of low virulence.

3. They are nonhemolytic and may or may not be green producers.

4. Biochemic and immunologic tests fail to show any constant identity between the individual streptococci concerned in producing this disease.

27. Jobling, J. W., and Petersen, W.: Jour. Am. Med. Assn., 1916, **66,** 1753.
28. Miller, J. L., and Lusk, F. B.: Jour. Am. Med. Assn., 1916, **66,** 1756.

5. The patients' blood serums contain agglutinating and complement fixing antibodies.

6. The reduction in the bacteriemia following transfusion with blood and impure saline depends on febrile reactions on the part of the patient. If such reactions do not occur, as when saline-free transfusions are used, no reduction in bacteriemia results. No noticeable benefit accrues in either case.

BACTERIOLOGIC STUDIES IN ACUTE RHEUMATIC FEVER*

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The present study was undertaken to determine whether any constant cultural or immunologic type of bacterium was associated with acute rheumatic fever. This disease presents a fairly typical clinical picture, consisting of an acute febrile condition, associated with polyarthritis, and a tendency to involvement of such serous surfaces as the endocardium, pericardium and pleura. Muscular tissues are also frequently involved, most noticeably the myocardium, in which a peculiar pathologic picture is commonly found in fatal cases. Certain symptoms of the disease, namely, the fever and polyarthritis, respond in a fairly characteristic manner to the administration of the salicylates in sufficient doses. It does not seem unreasonable, therefore, to suppose that the disease may be the result of the action of some definite pathogenic micro-organism.

The early contention of Achalme[1] that the disease was due to a gram-negative anaerobic bacillus has been disproved. The next view was that of Singer,[2] who claimed that the disease was an attenuated pyemia, due most frequently to the streptococci, but also to the staphylococci and other pyogenic organisms. Singer's contentions were based on bacteriologic studies of postmortem material and the isolation of pyogenic cocci from the urine. The sources of error connected with such studies are even more obvious today than they were to his opponents at that time. The extensive studies of Westphal, Wassermann and Malkoff[3] with a streptococcus which they isolated in a case of rheumatic fever, chorea and hyperpyrexia proved that a nonsuppurative polyarthritis could be produced in rabbits by a streptococcus. These authors attributed an etiologic rôle to the organism which they studied. Meyer[4] made cultures from the throats of acute rheumatic fever patients directly into broth. The injection of this

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3. Westphal, Wassermann and Malkoff: Berl. klin. Wchnschr., 1899, **36**, 638.
4. Meyer, Fr.: Verhandl. d. 19 Cong. f. inn. Med., 1901, p. 542.

mixed culture into rabbits was followed by a nonsuppurative arthritis; 10 per cent. of the animals had pericarditis or pleurisy and one fourth of the animals showed a verrucous endocarditis. From all of these lesions a diplostreptococcus which clouded broth could be isolated. The joint exudates soon became sterile and the animal recovered without permanent injury to the joint. Because cultures from normal throats, or from the angina of other disease, produced either suppurative arthritis, fatal septicemia, or no lesions at all, Meyer ascribed a specific etiologic rôle to these streptococci.

Menzer,[5] while confirming Meyer's results with streptococci from rheumatic angina, on the other hand was able to produce a similar arthritis and endocarditis with cultures from nonrheumatic or normal tonsils. He was unable to determine any colony, cultural or staining peculiarities in the streptococci from the tonsils of rheumatic patients compared with those from the tonsils of normal or nonrheumatic persons. He therefore concluded that "acute articular rheumatism is a peculiar clinical picture, not because there is a specific etiologic agent, but because the patient under certain inherited or acquired conditions and under acute or chronic influences reacts to the infection with common micro-organisms, apparently the organism found in the mouth, in a specific manner with manifestations which appear clinically as a specific illness."

Unfortunately, in none of these studies was any attention given to the hemolytic properties of the streptococci, so it is impossible to correlate absolutely the results with those of later workers. The evidence brought forward by Cole[6] and by Harris[7] to show that any streptococcus may produce destructive lesions of the joints likewise does not clear up this point, because the organisms studied were not divided into hemolytic and nonhemolytic forms. From our own studies and a review of the literature it is evident that the hemolytic forms may produce acute suppurative and destructive arthritis, provided they are not sufficiently virulent or injected in large enough doses to cause a fatal septicemia.

The work of Cecil[8] in this laboratory, however, conclusively demonstrates that destructive lesions of the articular surfaces often follow the intravenous injection of *Streptococcus viridans* into rabbits. The character of the exudate in this series was mucoid or mucopurulent. The exudate in the arthritis produced by hemolytic streptococci is frankly purulent.

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5. Menzer, A.: Die Aetologie des acuten Gelenkrheumatismus, Berlin, 1902.
6. Cole, R.: Jour. Infect. Dis., 1904, **1**, 714.
7. Harris, N.: Tr. Chicago Path. Soc., 1905, **6**, 303.
8. Cecil, R.: Jour. Exper. Med., 1916, **24**, No. 6.

In 1900 Poynton and Paine[9] gave the specific name *"Diplococcus rheumaticus"* to a diplostreptococcus which they isolated from four pericardial exudates, two blood cultures, two valve lesions and one angina, all from patients with acute rheumatic fever. Because they could demonstrate microscopically these organisms in rheumatic lesions, and produce similar lesions in experimental animals with pure cultures, they felt justified in applying a specific name to them. So thoroughly imbued are these authors with the specific nature of their diplococcus that they[10] apply the adjectives "rheumatic malignant" to the disease condition usually recognized as subacute streptococcus endocarditis. This they do because of the similarity of the micrococci associated with the two conditions. It is thus evident that their *Diplococcus rheumaticus* is similar to the *Streptococcus viridans* or *mitis*. Their cultural studies show that their organism is a diplostreptococcus giving a brownish color on blood agar plates, a turbidity in broth, and is gram-positive, though giving up the Gram stain more readily than do other gram-positive bacteria. The only point of difference from the ordinary *Streptococcus viridans* is in the size of the individual cocci, which they give as 0.5 microns. It is probable that the organisms measured by them were unusually small, such as are not infrequently encountered.

Another view of the rôle of streptococci in acute rheumatism is that of Rosenow[11] that the streptococci are constantly undergoing changes, assuming and discarding elective affinities for specific structures. It will be seen that this is the direct antithesis of Menzer's opinion, which places the onus on the patient's susceptibility to infection with mouth streptococci. This incomplete review of the literature gives a fair idea of the various opinions of the relation of streptococci to acute rheumatic fever.

While none of the classifications of streptococci are absolutely satisfactory, that proposed by Schottmüller[12] is almost universally accepted. In it we have hemolytic and nonhemolytic forms and, under the last group, those which form methemoglobin from the hemoglobin —the so-called viridans—and those which do not affect the hemoglobin, called anhemolyticus or saprophyticus by Le Blanc.[13]

A more extensive classification has been made by Andrewes and Horder,[14] based on the fermentation reactions of the streptococci.

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9. Poynton, F. J., and Paine, A.: Complete works collected in Researches on Rheumatism, London, 1913, p. 109.
10. Poynton, F. J., and Paine, A.: Complete works collected in Researches on Rheumatism, 1913, p. 312.
11. Rosenow, E. C.: Jour. Am. Med. Assn., 1915, **65,** 1687.
12. Schottmüller, H.: München. med. Wchnschr., 1903, **50,** 849.
13. Le Blanc: Centralbl. f. Bakteriol., Orig., 1911, **61,** 68.
14. Andrewes, F. W., and Horder, T. J.: Lancet, London, 1906, **2,** 708, 775, 852.

These authors recognized types which were associated to a certain extent with distribution in nature and pathogenic properties. While this classification has not been usually accepted, the study of fermentative reactions is of value and helps in a general way to place the organisms in groups.

In reviewing the various biochemical studies of streptococci, one occasionally meets with the results of studies made on organisms which have been isolated from patients with acute rheumatism. In trying to correlate these results one is struck with the diversity in types reported. Thus Andrewes and Horder[14] report that in the necropsies of fatal cases they secured two different main types, namely, *Streptococcus pyogenes* twice and *anginosus* variants seven times. They consider that these organisms may have been agonal invaders, because streptococci were only occasionally recovered from fatal cases. In testing a streptococcus rheumaticus from Poynton and Paine they found it to fall into the salivarius group and a *Micrococcus rheumaticus* sent by Beattie fell into the fecalis group. Beattie and Yates[15] report that three different streptococci isolated from the synovial membrane of the knee joint of acute rheumatic patients fell into the fecalis group. Floyd and Wohlbach[16] found two hemolytic streptococci, one, which clotted milk, fermented raffinose and salicin, hence fell into the *Streptococcus anginosis* group; the other did not change milk or the test sugars except lactose and fell into the pyogenes group. Still another one was nonhemolytic and fermented none of the sugars. Hopkins and Lang[17] found one nonhemolytic streptococcus which did not ferment any of the test objects. Lyall[18] reported on five streptococci, three isolated from the blood of rheumatics and two from the joints. All were indifferent to blood; one did not ferment either raffinose, inulin, salicin or mannite; one fermented only salicin, and three fermented salicin and mannite, which is characteristic of fecalis types. This small list proves that no single type of streptococcus has been constantly isolated in cases of acute rheumatic fever. Rosenow,[19] in reporting on the etiology of acute rheumatism, states that he was able to isolate three different types of organisms from the joint exudates. Tested on blood agar plates, they were, first, a green type forming long chains; second, a type giving slight hazy hemolysis about the colonies and forming short chains; all of Type 2 were mannite fermenters; third, a type with small grayish colonies indifferent to blood. In this paper Rosenow reported on his method of isolating

15. Beattie, J. M., and Yates, A. G.: Jour. Path. and Bacteriol., 1911, **16,** 247.
16. Floyd, C., and Wohlbach, S. B.: Jour. Med. Research, 1913, **29,** 493.
17. Hopkins, J. A., and Lang, A.: Jour. Infect. Dis., 1914, **15,** 63.
18. Lyall, H. W.: Jour. Med. Research, 1914, **30,** 487.
19. Rosenow, E. C.: Jour. Infect. Dis., 1914, **14,** 61.

the organisms from joint fluid by mixing the fluid in deep tubes of ascites dextrose agar, and from the blood by placing the sediment of centrifugalized laked blood in a similar medium. In this way streptococci were obtained from the joint fluid of fourteen out of sixteen patients; from the blood in three out of four.

We have studied the type of streptococci which we have been able to isolate from the blood of patients with acute rheumatic fever. In all fifty-eight cases have been studied. Nine were studied at the Hospital of the Rockefeller Institute three years ago and the remainder have been from the wards of the Presbyterian Hospital during the past two years. All were typical cases, showing fever and polyarthritis, and responded in the characteristic manner to salicylates. Blood cultures were made shortly after the admission of the patients to the hospital and before salicylates had been administered; also afterward at such times as complications or relapses appeared. The cultures were made from a few hours to several days after the appearance of the arthritis. The cultures of joint fluids were made at the same time, but in many of the cases it was difficult to obtain fluid from the affected joints. For this reason there were fewer cultures of joint exudates. The joints aspirated were knee, ankle, elbow and wrist.

Cultural Methods: In every case blood cultures were made as follows: (1) of from 2 to 3 c.c. of blood in dextrose agar plates; (2) by placing from 5 to 10 c.c. of blood in 125 c.c. of 1 per cent. dextrose broth; (3) by laking 5 c.c. of blood in 50 c.c. of sterile distilled water, centrifugalizing and placing the sediment in deep tubes of ascites dextrose agar. The cultures were usually made in duplicate. In addition, in many instances, laked sediment was placed on the surface of blood agar slants and in ascites dextrose broth.

In making cultures of joint fluid, deep tubes of ascites dextrose agar were mixed with increasing amounts of the fluid, in most cases 1, 2, 3, and 4 c.c. of fluid were placed in the different tubes. If there was sufficient fluid it was also put into deep tubes of ascitic dextrose broth and on the surface of blood agar slants. In this way all degrees of oxygen tension were obtained. The results are summarized in Table 1.

Altogether eighty-five blood cultures were made on fifty-eight patients with seven positive results, a percentage of 8.3. In one instance the cultures 59 *F* and *B* 38 were obtained from the same patient, so that in only six patients were positive cultures obtained. With the exception of this case, positive cultures were obtained only once in each patient and repetition of the cultures a short time afterward gave negative results. In three instances the cultures were

obtained at the time of an acute pericarditis. In two of these *A* 49 and 38 *D*, adults, previous cultures had been sterile, but at the time of the appearance of the pericarditis positive cultures were obtained, while later cultures were sterile. The other case of pericarditis was that of a child, 59 *F*, admitted to the Rockefeller Hospital in 1914. The culture was positive at the time of admission, but negative later. In 1916 she was readmitted to that hospital with typical acute rheumatism and polyarthritis; culture *B* 38 was obtained, but repeated attempts to secure a confirmation resulted in sterile cultures. Culture *B* 39 was from a child and *A* 65 and *A* 135 from adults with typical acute rheumatic fever.

All of the cultures of joint exudates have been sterile. Joints have been aspirated within four hours after the appearance of the exudate and at various later intervals. As positive blood cultures have been obtained from patients at the time that joint exudate cultures were sterile, it is evident that the medium was suitable for the growth of the streptococci. We therefore do not feel that the difference between our results and those of Rosenow can be explained by technical errors, such as age of the arthritis or faults with culture mediums. In addition all of the joint exudates have been examined microscopically and in no instance have we found diplococci or streptococci in smears.

TABLE 1.—Results of Blood and Joint Cultures

	Blood Cultures			Joint Cultures		
	Number of Patients	Number of Cultures	Number of Positive Cultures	Number of Patients	Number of Cultures	Number of Positive Cultures
Adults.....	44	60	4	23	31	0
Children.....	14	25	3	2	3	0
Total.....	58	85	7 (8.2%)	25	34	0

Patients have also been studied in whom arthritis was not present. These include principally cases of endocarditis with fever and accompanying conditions, such as pleurisy, pericarditis or chorea. While it is generally considered that these are rheumatic manifestations, it was thought better to place them under a separate heading. In two such instances streptococci were obtained in blood culture. Culture *A* 119 was obtained from a patient during a febrile attack accompanied by pain in the knee, but no other arthritis symptoms. Culture *A* 141 was obtained from a child with pericarditis and endocarditis during an attack of "decompensation" and pleurisy. Subsequent cultures were

sterile, but the "rheumatic" nature of the condition was demonstrated a year later at necropsy. In addition, three cases of acute rheumatic pericarditis and two of endocarditis gave sterile blood cultures during the acute period of the disease. The aspirated pericardial fluid from one of the pericarditis patients also yielded negative cultures.

Necropsies: The difficulty of obtaining a pure culture at necropsy is almost insurmountable and hence the results of necropsy cultures are inconclusive. Six fatal cases have been studied. Three of these patients had acute pericarditis and acute endocarditis, two acute endocarditis and one chorea and endocarditis with terminal pneumonia.

TABLE 2.—RÉSUMÉ OF POSITIVE CULTURES

Number	Day of Disease after Onset of		Mediums in Which Positive Cultures Grew*	Joint Cultures
	Arthritis	Pericarditis		
A 65	4th	0	Laked sediment in ascites dextrose agar tube only	Sterile
A 135	11th	0	All mediums	Sterile
B 39	4th	0	Two broth flasks, no others made	Not made
59 F	1st attack 9th	1st	One broth flask only	Not made
B 38	2d attack 2d	....	All mediums; 100 colonies per c.c.	Sterile
38 D	12th	....	Sterile	
	15th	1st	Laked sediment in ascitic broth only	Sterile
A 49	14th	....	Sterile	
	19th	1st	Colonies in only 1 dextrose blood agar plate	Not made
A 119	Indeterminate 2d day of febrile attack	Previous attack	Colonies in only 1 dextrose blood agar plate	Not made
A 141	Indeterminate during pleurisy	Previous attack	Laked sediment in 1 ascitic dextrose agar tube only	Not made
A 178	Necropsy	2½ mo.	Blood agar plates	

* Positive cultures developed in all mediums in two cases; laked sediment in deep ascitic dextrose agar tubes only, in two cases; laked sediment in ascitic broth only, one case; blood broth flask only, one case; both blood broth flasks, one case; dextrose blood agar plates only, two cases.

From the vegetations of one of the endocarditis cases streptococci were grown in pure culture and from the other a mixed culture of staphylococci and diphtheroid bacilli. From the heart's blood of the chorea and endocarditis patient, a Group 4 pneumococcus was obtained. In the cases of pericarditis and endocarditis the cultures from endocardial lesions in one case yielded a staphylococcus and a green-forming streptococcus which fermented raffinose. From the second case, in which the heart was opened under rigid precaution after removal from the necropsy room, three types of cocci were

obtained, (1) pneumococcus, (2) hemolytic streptococcus, and (3) a green-forming streptococcus. The streptococci from both these cases were lost before they were completely studied. In the third case *A* 179, diphtheroid bacilli and green streptococci were obtained from all the diseased valves. The characteristics of these cocci are given under the subsequent studies. The case reports are given at the end of the paper.

Cultural Characteristics.—The cultural characteristics and virulence of the organisms have been studied immediately on isolation. All were gram-positive cocci, with a tendency to grow in pairs or short chains. Plain broth cultures were turbid and did not clear on the

TABLE 3.—CULTURAL AND VIRULENCE CHARACTERISTICS

No.	Growth in		Bile Solubility	Effect on Red Blood Cells	Fermentation Reactions					Lethal Dose for White Mice
	Bouillon	Ascitic Dextrose Agar			Milk	Raffinose	Inulin	Salicin	Mannite	
B 38	Turbid	Marked clouding	Insoluble	Green	+	+	+	0	0	2 c.c. 0
A 179	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	2 c.c. +
B 39	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	2 c.c. 0
59 F	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	0.1 c.c. +
A 49	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	0.1 c.c. +
A 141	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	2 c.c. +
A 119	Slightly turbid	Marked clouding	Insoluble	Slightly green	+	0	+	+	0	2 c.c. 0
38 D	Slightly turbid	Marked clouding	Insoluble	Green	+	0	0	+	0	1 c.c. +
A 135	Turbid	Marked clouding	Insoluble	Green	+	0	0	+	+	2 c.c. 0
A 66	Turbid	Marked clouding	Insoluble	Green	+	+	0	0	0	0.1 c.c. +

addition of equal parts of ox bile. No capsules could be demonstrated in the smears of the peritoneal exudates of mice inoculated intraperitoneally with the organism. The action on blood was studied, both on human blood plain agar plates and by mixing diminishing dilutions of twenty-four hour plain broth cultures with equal parts of 5 per cent. suspension of sheep's blood cells and incubating two hours. None of the strains produced hemolysis of the cells or clearing about the colonies on the blood agar plate and all turned the cells brown and gave a green appearance on the plates. This is characteristic of the so-called *Streptococcus viridans.*

The fermentation reactions were studied with milk, raffinose, inulin, salicin and mannite in Hiss serum water mediums. These mediums were heavily inoculated with an actively growing twenty-four-hour broth culture, and observed over a period of ten days. The fermentation reactions were studied at the time of isolation of the culture, and again in June, 1916. None of this series underwent any change during the interval. All the strains produced acid and clot in milk. Five of the strains did not ferment any of the test objects. The other five showed individual fermentative capacity, no two falling into the same group. Only one was a mannite fermenter, thus placing only one of our series in the fecalis group.

The virulence was tested by injecting 2 c.c. 1 c.c. and 0.1 c.c. of a twenty-four-hour plain broth culture intraperitoneally into white mice. The lethal dose of three of the strains was 0.1 c.c., of one it was 1 c.c., of two it was 2 c.c., and four were not fatal in 2 c.c. quantities. Only in the point of virulence have organisms in this group shown peculiarities. Compared with green-producing streptococci from normal mouths or from patients with subacute bacterial endocarditis most of these strains have been distinctly virulent, although compared with hemolytic streptococci or pneumococci, their virulence is low. On cultivation the original virulence has been fairly well maintained, and repeated passage through mice has failed to increase the virulence to any appreciable degree.

Immunologic Classification.—In addition to a study of the cultural characteristics, an attempt has been made to determine whether there was any immunologic grouping. For this purpose the complement fixation reaction has been used, employing antiformin solutions of the various streptococci as antigens. The exact method is fully described elsewhere.[20] Rabbits were injected intravenously with killed streptococci until their serum gave good fixation with its homologous antigen. It was then tested for its complement fixing power against antigens prepared from all the streptococci. The results are summarized in Table 4.

It will be seen that no single group exists. One member, *A* 65, is not related to any of the others. Among the remaining strains roughly three groups can be made out. The first consists of three strains, *B* 38, *A* 179 and *B* 39; the second of 59 *F* and *A* 49, which appear to be almost identical; and a third, which consists of *A* 141, *A* 119 and 38 *D*. The first and second groups are connected by serum *A* 141, which also fixes antigen *A* 135. Comparing the individual strains, however,

20. Kinsella, R. A., and Swift, H. F.: A Classification of Nonhemolytic Streptococci. To be published.

we find that the only two which appear to be very closely related are 59 *F* and *A* 49.

It is not the intention to discuss the general subject of the classification of streptococci by means of the complement fixation reaction, as that is more fully considered in another communication. The evidence brought forward in this table is merely to show the immunologic relationship of the various strains.

A comparison of the fixing properties with the fermentation reactions and virulence of the several strains shows that no strict relationship exists between the various fermentation groups and the groups as determined by complement fixation. In the study of the virulence of the strains no special tendency toward virulence is noted among any one of the various fermentation groups or complement fixation groups,

TABLE 4.—GROUPING BY MEANS OF COMPLEMENT FIXATION REACTIONS *

Serum of Rabbits Immunized With	Antigens										
	B 38	A 179	B 39	59 F	A 49	A 141	A 119	38 D	A 135	A 65
B 38	+	−	±	−	−	−	−	−	−	−
A 179	−	+	±	−	−	−	−	−	−	−
B 39	+	+	+	+	±	−	−	−	−	−
59 F	−	−	−	+	+	−	−	−	−	−
A 49	±	±	−	+	+	−	−	−	−	−
A 141	−	−	−	+	+	+	+	+	+	−
A 119	−	−	−	−	−	−	+	+	−	−
38 D	−	−	−	−	−	−	−	+	−	−
A 135	−	−	−	−	−	−	−	−	+	−
A 65	−	−	−	−	−	−	−	−	−	+

* In this table + signifies that fixation with heterologous antigen was of practically the same strength as with homologous antigen; ± signifies that fixation with heterologous antigen was weaker than with homologous antigen; − signifies negative reaction.

with the exception of 59 *F* and *A* 49, which seem to be closely related in all their characteristics. Compared with a larger number of green-forming streptococci obtained from blood cultures of *Streptococcus viridans* from endocarditis patients and from the throats of acute angina patients, the streptococci from acute rheumatic fever patients could not be differentiated by means of cultural characteristics. In the immunologic studies most of the streptococci from rheumatic fever patients fell in one side of the large group, although various individual members are scattered throughout the group. In the matter of virulence six out of ten rheumatic fever strains were virulent, while with one exception all the endocarditis strains were avirulent. On the other hand, angina strains were often virulent to 1 c.c. or less.

Study of Antibodies in Patients' Serum.—After it was shown[21] that the serum of patients with subacute streptococcus endocarditis contained antibodies against homologous strains, it was thought that antibodies might be present in the serum of acute rheumatic fever patients whose blood yielded a positive culture. Likewise, if a specific *"Streptococcus rheumaticus"* existed, the serums of rheumatic fever patients might show specific antibodies. These antibodies were tested for by means of the thread reaction, a modification of the agglutination reaction which has proved more satisfactory with streptococci than the ordinary agglutination reaction. The serums of five patients tested failed to react to their respective streptococci. The serums of twelve other rheumatic fever patients failed to react with any of the four strains, *A* 49, 59 *F*, 38 *D* and *A* 65, a result which is easily explained by the individuality of these strains. The failure of serums to react with homologous strains is, however, most striking when compared with the results obtained in a type of disease such as subacute streptococcus endocarditis, in which we are certain of the relationship between the bacterium and the disease.

COMMENT

We do not feel that our results clear up the much-vexed question of the relation of the nonhemolytic streptococcus to acute rheumatic fever. From a statistical point of view the small percentage of positive cultures would incline toward the negative side of the question. Streptococci are found in the blood of scarlet fever patients with an equal frequency, and yet most observers do not believe that the streptococcus is the cause of scarlet fever, but rather that this organism acts as a secondary invader; and it is conceivable that it may play a similar rôle in acute rheumatic fever. The relatively high frequency with which the streptococcus was demonstrated in patients with complications such as pericarditis or pleurisy lends support to this opinion. Again, the failure to demonstrate constantly the streptococcus in the verrucae of acute rheumatic endocarditis is an argument against the specific etiologic relationship of this organism.

On the other hand, a small proportion of positive cultures may simply mean that the streptococci circulate in the blood stream only for short periods, or are rapidly killed off, so that the methods employed are sufficient to demonstrate them only occasionally. Furthermore, our studies of the cultural and immunologic characteristics show that the streptococci which are present in this disease are merely various members of the *Streptococcus viridans* group, and should not be separately classified as *Streptococcus rheumaticus*. Of extremely

21. Kinsella, R. A.: THE ARCHIVES INT. MED., this issue, p. 367.

important bearing on this question are the findings in cultures 59 *F* and *B* 38. At the time of the first attack, streptococcus 59 *F* was isolated. After a prolonged course the patient recovered from every evidence of acute or subacute infection. After a year of good health she suffered a relapse, or as is more probable, a new infection. At this time streptococcus *B* 38 was recovered from her blood. This proved to be an organism that differed culturally, immunologically and in its virulence from the strain originally isolated. In addition, no complement fixing or agglutinating antibodies against 59 *F* could be demonstrated in the patient's blood at the time of the second infection. If the infection was a chronic one due to streptococcus 59 *F*, we would expect to find antibodies such as may be demonstrated in the serum from cases of subacute streptococcus endocarditis. We feel that more detailed observation and experimentation is required to decide the points at present under discussion.

Another point should be mentioned: There is a tendency among some workers to consider that because a green streptococcus is isolated from the blood of a patient with endocarditis, there is sufficient ground for classifying the case in question as subacute bacterial endocarditis —the type so well described by Libman. This disease, with its characteristic symptomatology, is now generally recognized as a fatal malady. From our studies we feel that the essential factors in its recognition do not depend alone on the isolation of the infecting organism, but on such characteristic symptoms and signs as petechiae, embolic lesions and progressive downward course, in addition to the picture of an endocarditis. The findings in our cases indicate that green streptococci can be isolated from the blood stream, and still the patient may not suffer from subacute bacterial endocarditis. Some of the patients have been followed for a year or more without showing any signs of this disease, and case *A* 141, a year after yielding a single positive blood culture, showed at necropsy characteristic rheumatic lesions in the heart valves and muscle.

SUMMARY AND CONCLUSIONS

1. Cultures of the exudate aspirated from the joints in acute rheumatic arthritis have been uniformly sterile.

2. Nonhemolytic streptococci have been recovered in blood culture from less than 10 per cent. of patients suffering from acute rheumatic fever.

3. Similar streptococci have been recovered from the active endocardial lesions in only half of the fatal cases of acute rheumatic fever.

4. From the above results it seems evident that no type of streptococcus has been constantly associated with acute rheumatic fever.

5. We do not feel that the etiologic relationship between the streptococcus and acute rheumatic fever has been definitely proved, but if the streptococcus is the etiologic agent in acute rheumatic fever, it is shown by means of cultural and immunologic studies that it is through various members of the viridans group, and hence no one member can be called the *Streptococcus rheumaticus*.

CASE REPORTS

A 65.—J. C., a man aged 19, a painter, was admitted to the Presbyterian Hospital Jan. 8, 1915, and discharged February 7. On January 9, herniotomy was performed. The temperature was normal four days and then it ranged between 99 and 103 for seven days. On the 19th blood cultures made in broth flasks were sterile. On the 25th the patient suffered pain in both ankles and the right knee. On the 27th there were general malaise and chilly sensations, the joints being swollen and tender. Blood culture in broth flasks was sterile. On the 28th blood cultures in broth flasks and ascitic dextrose agar plates were sterile. On the 29th blood cultures were positive for *A* 65. Cultures were developed only in laked sediment in ascitic dextrose agar tube. A culture of fluid from the right knee was sterile. On the 31st extension of arthritis to wrist and hands followed. Prompt relief was obtained from 100 grains of sodium salicylate per day. On February 7 the patient insisted on going home because he felt so well. The serum gave no thread reaction with *A* 65 culture.

A 135.—O. C., a man aged 26, a tile-layer, was admitted to the Presbyterian Hospital April 7, 1915, and discharged on May 11. He had suffered six years before with "acute rheumatism," involving all the joints. He was ill four months five years before with a similar attack, his illness lasting one month. His present illness began two weeks before with "cold in head and chest." Eleven days before his left knee became swollen, tender and painful; this was followed by the involvement of all the joints of the arms and hands and the right knee. There was very little general illness. Physical examination revealed pyorrhea. There was an abscess at the root of the first right lower molar. The heart was not enlarged, but there was a faint murmur at the apex not transmitted and at the base. There was general polyarthritis. On April 7 a blood culture proved positive for *A* 135. Growth took place in all media. The right knee yielded 20 c.c. of murky, lemon-colored fluid, the left 15 c.c. of similar fluid. Cultures of this fluid were sterile. Fever was absent after three days of salicylates, and the joints returned to normal after five days. The patient was discharged without any complications of the disease.

B 39.—B. M., a girl, aged 6 years, was admitted to the Presbyterian Hospital March 10, 1916, and discharged April 9. The family history showed that the mother and one sister had syphilis. The child had had some joint trouble when a few weeks old. She had acute rheumatism eighteen months before admission and numerous attacks of tonsillitis, the last being one month before. Three days before there was pain in both wrists, lasting two days, and one day before the ankles became painful and swollen. Physical examination showed the tonsils much enlarged, not acutely inflamed. The heart was enlarged to the left and downward. There was a systolic murmur at the apex. A diastolic murmur was perceptible over the aortic area. Both ankles were hot, tender and swollen. On March 11 a blood culture in two flasks showed both positive for *B* 39. On the 14th a blood culture proved sterile. The arthritis cleared up without salicylates. On April 7 a blood culture was sterile and the general condition was good.

59 *F* and *B* 38.—F. B., a schoolgirl, aged 8 years, was admitted to the Rockefeller Institute Hospital May 26, 1914, and discharged June 2, 1915. The child was suffering with rheumatic fever, acute pericarditis, valvular disturbance and mitral insufficiency. She complained of pain over the heart. Two weeks previously the mother and one brother of the patient had sore throat, but the patient did

not. The patient had measles at 4, but no scarlet fever. She often had bronchitis, but no sore throat. At 1 year there was twitching of the left arm (chorea). She never had pain in the joints. Eleven days previously the patient fell while skating. Ten days before she vomited and had headaches. Nine days before her feet hurt and the right one was swollen. Eight days before both ankles and knees were swollen and painful. This gradually decreased. Her general condition improved from day to day. The day before entrance she was out of bed, and on the day of entrance she was attacked with a sudden pain in the region of the heart. Physical examination revealed dyspnea, painful expression and a dry cough. There was a friction rub over heart. The heart was 4 cm. to the right and 11 to the left of the midsternal line. The liver was enlarged, extended 3 cm. below the costal margin and was tender. There was no edema. The joints were negative. Blood culture was positive for 59 *F*. Growth took place only in the broth flask. The friction rub persisted until June 8. Fever lasted until July 12. From September 21 to 29 there was fever, but the blood cultures were sterile. On Aug. 7, 1915, the tonsils were removed.

The patient was readmitted to the hospital March 9, 1916. She had remained well until one month before, when she had grip and fever for three days. Three weeks before she had grip for two weeks, with high fever; then she was well one week. Two days before entrance she vomited and had headache. The day before she had pain in both ankles. Examination showed no petechiae. The throat was clear. The heart was enlarged, and there was an apical systolic murmur. There was heat and tenderness in the left ankle. A blood culture was positive for *B* 38, giving 100 colonies per cubic centimeter, which developed in all mediums. February 12 both knees were swollen and tender. March 10 the ankles, knees, elbows and wrists had been involved. Blood cultures on March 11, 12 and 13 were sterile. On the 12th 5 c.c. turbid fluid were aspirated from the knee joint, but a culture proved sterile. There was no trace of petechiae. The fever ranged from 101 to 102 until May 5.

38 *D*.—J. McK., a blacksmith, aged 58, was admitted to the Rockefeller Institute Hospital March 15, 1914, and discharged June 9, 1914. There was a history of acute rheumatism four years previously. There had been no cardiac symptoms afterward. Two weeks before entrance he caught cold, and eleven days before his left wrist became red and swollen. Five days before his knees became involved, and four days before his right wrist also was affected. He was confined to bed for two days. Examination showed the heart to be slightly enlarged. There was an apical systolic murmur. There was acute arthritis in the right wrist, both elbows, the knees and the ankles. On March 16 a blood culture was sterile, and the fluid from both knees and ankles was sterile. On March 19 there was a pericardial friction rub. A blood culture was positive for 38 *D*, developing from laked sediment in ascitic broth. On March 20 a blood culture was sterile. On the 21st there were signs of fluid in the left pleural cavity. On the 26th the pericardial rub had disappeared and the blood culture was sterile. From March 29 to April 11 there was continuous auricular fibrillation. On April 12 the heart showed sequential rhythm. May 9 the patient showed a slow but steady convalescence.

A 49.—A. G., a waitress, aged 23, was admitted to the Presbyterian Hospital Jan. 16, 1915, and discharged April 6, 1915. Eleven years before the patient had a severe acute articular rheumatism, followed by chorea. The present illness began two weeks before with polyarthritis, involving both arms and legs. Later this had receded, but there were fever and sweating, and the previous few days dyspnea. Examination showed the heart to be enlarged, and there was an apical systolic murmur. The left wrist was swollen, red and tender. On January 16 blood culture proved sterile. From the 17th to the 19th the wrists, shoulder and knees were involved. On January 21 there was a pericardial rub.[22] Blood

22. During the period from Jan. 16 to 23, 1915, the patient was taking sodium salicylate from 100 to 200 grains per day.

culture was positive for *A* 49, colonies arising in one blood agar plate. This was followed by effusion into the pericardium, which persisted until the middle of February. On February 1 she had a febrile attack without new symptoms, but a blood culture was sterile. On the 3d there was a fresh effusion into the knee joint, but a culture was sterile. Convalescence was slow but steady until the patient was discharged April 1.

In the spring of 1916 the patient reported another attack of acute rheumatic fever without cardiac involvement. In May, 1916, the blood serum gave no complement fixation with antigen *A* 49.

A 119.—H. F., a boy, aged 16, a clerk by occupation, was admitted to the Presbyterian Hospital March 2, 1915, and discharged April 9, 1915. The boy had attacks of precordial pain and dyspnea in 1909, 1910, 1911 and 1912. In 1912 he had some joint pains. In 1913 in this hospital a diagnosis was made of acute simple endocarditis, fibrinous pericarditis and left hydrothorax. There were double murmurs in both mitral and aortic areas. The boy was well and working until two weeks before entrance. He had cough two weeks, with increasing dyspnea, swelling of abdomen and legs. A physical examination showed dyspnea, orthopnea, with the throat clear, except for enlarged tonsils. The heart was much enlarged to the right and left. There were double mitral and double aortic murmurs, congestion of the lungs, enlarged liver, ascites and edema of the legs. On March 5 the patient had an attack of pulmonary edema. On the 9th steady improvement was apparent. March 14 to 16 the temperature was 102. There were precordial pain and pain in the left knee, but no swelling or redness. On March 16 a blood culture was positive for *A* 119, colonies developing on blood agar plate. There was immediate improvement after taking salicylates. April 5 the temperature was normal, and the patient showed steady improvement. A blood culture was then sterile. The boy was discharged April 9, and he returned to work.

A 141.—J. K., a schoolgirl, aged 10 years, was admitted to the Presbyterian Hospital Oct. 3, 1914, remaining till Dec. 29, 1914. She was readmitted April 7, 1915, and remained till June 16, 1915. She had diphtheria at the age of 5 years. She was always subject to sore throat. In April, 1914, she suffered from dizziness and vomiting. A diagnosis of rheumatic heart was made. This cleared up with rest. On Oct. 3, 1914, there were dizziness, cyanosis and dyspnea for three days. Examination showed the heart to be enlarged. An apical systolic murmur was transmitted over the entire chest. There were congestion of the lungs and enlarged liver. Fever of 103 was present, with respiration 56. On October 7 a blood culture showed three plates sterile. On the 12th a blood culture of two plates of dextrose blood agar was sterile. From the 14th to the 24th a precordial friction rub was perceptible. On the 29th there was general edema. November 24 the patient showed steady improvement, but there was fever of 101 each day. A blood culture on four dextrose blood agar plates was sterile. On December 22 the patient was much improved and was discharged.

She was readmitted April 7, 1915, complaining of increasing anorexia, nausea, pain in the chest and coughing. The throat was clear. The heart was enlarged, and showed a systolic retraction over the precordium. There were systolic and presystolic murmurs at the apex. The lungs were congested and a friction rub over the right base posteriorly was discernible. On April 13 a blood culture was positive for *A* 141, colonies developing only in laked sediment in ascites dextrose agar tube. On April 19 and May 20 blood cultures were sterile. June 1 the patient showed a steady convalescence, but she had a temperature of 101 nearly every day. On June 16, 1916, she was readmitted in extremis. A blood culture was sterile.

Necropsy showed adherent pericardium, acute vegetative endocarditis of verrucous type, chronic cardiac valvular disease, chronic mural endocarditis, adherent pericardium, pleuritic adhesions (both sides) and rheumatic myocarditis.

A 179.—A. B., a schoolgirl, aged 12 years, was admitted to the Presbyterian Hospital Feb. 26, 1915, and died May 20, 1915. She had acute rheumatism four

years before, lasting three weeks. Two weeks before entrance there was pain in the legs; then the shoulders, ankles and knees became involved. There was pain over the precordium for several days, increasing dyspnea and weakness. Examination showed the pharynx to be diffusely reddened and the tonsils large. The heart was enlarged to the left and downward. There was a distinct gallop rhythm, with systolic and diastolic murmurs at the apex. The liver was enlarged, and there was tenderness over it. The right hand, left ankle and right knee were tender, painful and swollen. February 27 a blood culture was sterile. On March 3 the area of cardiac flatness was much increased. The cardiohepatic angle was obtuse and the heart sounds muffled. From March 17 to April 12 the patient had attacks of slowing of the pulse in which the rate was one-half the ordinary rate. Electrocardiograms showed sudden alternating transition of auricular rate from 53 to 108. At both rates the conduction mechanism was normal. From April 14 to 18 there were precordial friction rubs, and also from May 4 to 7. On May 14 there was much precordial pain, with chorieform movements. May 20 the condition steadily grew worse until death.

Necropsy showed acute rheumatic endocarditis, involving the mitral, aortic and tricuspic valves and foramen ovale; acute pericarditis, adherent pericardium; rheumatic myocarditis; many Aschoff bodies; cardiac hypertrophy; rheumatic pleurisy; mediastinitis; petechial hemorrhages into the fatty tissue of the abdomen, the pectoralis minor muscles and the pericardium, and beneath the endocardium in the right auricle; acute hemorrhagic enteritis. Cultures showed the hemorrhagic area from the left pectoralis minor muscle to be sterile. The heart's blood showed three diphtheroid colonies. The valve lesions were cultured and diphtheroid and green forming streptococci *A* 179 developed from each valve, including the tricuspid valve, the mitral valve and the aortic valve.

THE MALONE-KIUTSI REACTIONS IN PREGNANCY AND CANCER*

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Various attempts have been made to simplify the technic of the Abderhalden dialysis test, but without success. Some of the investigators have applied the technic originally described by him with the use of the urine instead of the blood serum. The results have been variable.

The theoretical basis for the use of the urine has been on the supposition that the enzymes which dialyze through a parchment thimble pass through the kidney and are found in the urine (Berman,[1] Malone[2]).

L. M. Warfield[3] had reported seventeen analyses (Abderhalden method) on urines of pregnant women in which he had obtained positive reactions in all. Fischer[4] pointed out later that Warfield[3] had omitted the urine of males and nonpregnant females as controls. Fischer[4] found that in his laboratory all urines with few exceptions gave a positive ninhydrin reaction. These findings coincide with my observations. He felt that the negative reactions were due to some ninhydrin inhibiting substances, but he did not attempt to explain their nature.

Berman[1] had reported the dialysis test on ninety-seven urines of pregnancy, forty-eight specimens of gynecologic patients and ten of males. His positive reactions to ninhydrin after incubation were 92, 89, and 70 per cent., respectively.

Falls and Welker[5] in a detailed report stated that amino-acids form an appreciable part of the normal nitrogenous elimination in the urine. They reasoned that if ninhydrin reacted with compounds containing the intact amino group, then all normal urines should react positively with the reagent unless the content of the amino-acids fell below the limits of the delicacy of the reaction.

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1. Berman, L.: Application of the Ninhydrin Reaction to the Urines and Urinary Dialysates of Pregnant Women, Am. Jour. Obst., 1914, **70,** 192.
2. Malone, R. H.: Jour. Am. Med. Assn., 1915, **64,** 1651.
3. Warfield, L. M.: Jour. Am. Med. Assn., 1914, **62,** 436.
4. Fischer, C. E. M.: Jour. Am. Med. Assn., 1914, **62,** 950.
5. Falls, F. H., and Welker, W. H.: Jour. Am. Med. Assn., 1914, **62,** 1800.

In June, 1914, M. Kiutsi[6] of Japan claimed to have discovered a new modification of the Abderhalden reaction in which he had avoided using dialyzing thimbles, with excellent results. In pregnancy he had "repeated this method hundred times and never missed." Moreover, he stated in a later communication[7] that "it is not at all difficult with the improved method to diagnose the pregnance in, but two hours after the test began."

He claimed that he could diagnose cancer, tuberculosis, gallbladder disease, appendicitis, retinitis, and choroiditis, and had isolated a ferment for each disease. In other words, a large number of our present common diseases could be diagnosed by laboratory methods alone.

One is impressed in a perusal of his reports by the secrecy of the details of the technic in such an important discovery. In his test for pregnancy he made use of a preparation, called by him "ninserin," as the substrate, but he did not give the details of the preparation.

In view of Malone's results in pregnancy, I made attempts to corroborate his findings and extend the usefulness of the test to cancer. This study covers sixty-seven analyses, including urines of pregnant and nonpregnant women, urines of cancer patients and urines from normal males. The results are discussed in the accompanying tables.

I undertook to determine the following:

1. The preparation of the substrate.
2. The preparation of the urine.
3. The technic of the test.
4. The determination of the period of incubation (Table 1).
5. The value of the test in pregnancy (Table 2).
6. The value of the test in cancer (Table 3).
7. The reaction of the urine of pregnant and cancer patients on both placental and cancer substrates (Tables 3 and 4).

1. Preparation of the Substrates: For the pregnancy test fresh placenta was used, while for the cancer test an adenocarcinoma of the liver was employed. The preparation in both instances was alike.

A fresh placenta was placed in normal saline solution and as much blood forced out of it as possible. It was then divided into small pieces with an ordinary meat chopper, after discarding all the blood vessels and connective tissue, and placed into running tap water for approximately thirty-six hours. The residual tissue appeared snow white. All red pieces and blood clots were removed before boiling in distilled water. Five drops of dilute acetic acid were then added to

6. Kiutsi, M.: Kiutsi's Urindiagnosis by Means of "Filtration Process," June 10, 1914, printed by Bunyeido, Sapporo, Japan.

7. Kiutsi, M.: Kiutsi's Isolation of Protective Ferments and Progress of Filtration Process, Oct. 1, 1914, printed by Bunyeido, Sapporo, Japan.

the water, as recommended by Abderhalden. It was boiled for five minutes and then washed with distilled water four or five times by shaking with water equal to five times the amount of substrate used; the supernatant fluid was decanted each time. The boiling process was repeated till 5 c.c. of filtered decanted water gave a negative reaction with 1 c.c. of 0.1 per cent. ninhydrin in solution (acetic acid was added only in the first boiling).

The next step consisted in drying the substrate in a water chamber oven at 80 C. for four hours and over night at 55 C., and then in a desiccator over calcium chlorid for three days. The resulting brown powder was very dry and hard, and was kept in a ground glass stoppered bottle in a dark place. The substrate used in the cancer tests was prepared in the same way.

TABLE 1.—Determination of the Period of Incubation *

Specimen Number	C.c. Each Test	2 Hrs.		6 Hrs.		12 Hrs.		16 Hrs.		24 Hrs.	
		B.	N.	B.	N.	B.	N.	B	N.	B.	N.
A 1	5	0	+	0	+	0	++	0	++	0	++
C 2	5	0	+	0	+	0	+	0	++	+	++
C 3	5	0	+	0	+	0	+	+	++	++	++
A 4	5	0	+	0	++	0	++	0	++	++	++
A 5	5	0	+	0	+	0	++	0	++	++	++
C 6	10	0	+	0	+	0	++	0	++	++	++
C 7	10	0	+	0	+	0	+	0	++	++	++
C 8	5	0	+	0	++	++	++	++	++	++	++
A 11	10	0	+	0	+	0	+	0	+	++	++
A 12	5	0	+	0	+	0	+	0	+	++	++

* In this and the following tables B, signifies biuret test; 0, negative; + positive; N, ninhydrin test; C, pregnancy urines; and A, the urine from males. All urines were incubated at 37.5 C.

2. Preparation of the Urine: The urines were obtained immediately after voiding and examined for albumin by the biuret test. If positive, they were made negative by the following method: Fifteen cubic centimeters of urine were placed in a well-stoppered bottle and shaken for ten minutes with 0.3 gm. kaolin; it was then filtered, and tested again. The shaking process was repeated till the biuret test was negative. Attempts to shake the urine with animal charcoal obtained from several different sources were not satisfactory, inasmuch as it was necessary to keep increasing the amount of urine used and a longer time was consumed to render the urine negative. Ten c.c. of the negative urine was neutralized to litmus with 2 per cent. sodium carbonate solution, if acid, or with 1 per cent. acetic acid, if alkaline.

3. The Technic of the Test: Ten c.c. prepared urine and 0.2 gm. of

TABLE 2.—THE VALUE OF THE TEST IN PREGNANCY *

Specimen Number	Diagnosis	C.c. Each Test	Hours Incubation	Control (1) Urine		Urine and Placenta		Control (2) Urine	
				B.	N.	B.	N.	B.	N.
1	Male—pneumonia...	5	12	0	+	0	++	0	+
2	Pregnancy.....	5	16	0	++	0	++	0	++
3	Pregnancy.....	5	16	0	+	+	++	+	++
4	Male—nephritis.....	5	12	0	++	0	++	0	++
5	Male—carditis.....	5	12	0	++	0	++	0	++
6	Pregnancy.....	5	12	0	+	0	++	0	++
7	Pregnancy.....	5	12	0	++	0	++	0	++
8	Pregnancy.....	5	12	0	++	++	++	0	++
9	Pregnancy.....	5	16	0	+	0	+	+	++
10	Pregnancy.....	5	16	0	+	0	+	+	++
11	Male—nephritis.....	10	16	0	+	0	++	+	++
12	Male—nephritis.....	10	16	0	+	0	+	0	+
13	Pregnancy.....	10	16	0	+	0	+	++	++
14	Pregnancy.....	10	16	0	++	++	+	0	++
15	Pregnancy.....	10	16	0	+	+	++	0	++
16	Pregnancy.....	10	16	0	+	0	++	0	++
17	Pregnancy.....	10	16	0	+	0	+	+	+
18	Pregnancy.....	10	16	0	+	+	++	0	+
19	Male—pneumonia...	10	16	0	+	0	+	+	++
20	Pregnancy.....	10	16	0	+	0	+	+	++
21	Pregnancy.....	10	18	0	+	0	++	0	++
22	Pregnancy.....	10	18	0	+	0	++	++	++
23	Pregnancy.....	5	24	0	+	++	++	+	++
24	Pregnancy.....	5	24	0	+	+	++	+	++
25	Pregnancy.....	10	24	0	+	+	++	++	++
26	Male—healthy.....	10	24	0	+	+	++	+	++
27	Pregnancy.....	10	24	0	+	0	+	+	++
28	Pregnancy.....	10	24	0	+	+	++	+	++
29	Pregnancy.....	10	18	0	+	0	+	0	+
30	Pregnancy.....	10	18	0	+	+	+	+	+
31	Pregnancy.....	10	18	0	++	+	++	0	++
32	Pregnancy.....	10	18	0	+	+	++	++	++
33	Pregnancy.....	10	18	0	+	0	+	+	++
34	Male—healthy.....	5	18	0	+	0	+	+	+
35	Female—Ca. breast; not pregnant.....	5	18	0	+	0	++	+	++

* In addition to the symbols explained in the footnote to Table 1, in this and the following tables Ca. signifies carcinoma; (1), before incubation; (2), after incubation.

TABLE 2.—THE VALUE OF THE TEST IN PREGNANCY*—(*Continued*)

Specimen Number	Diagnosis	C.c. Each Test	Hours Incubation	Control (1) Urine		Urine and Placenta		Control (2) Urine	
				B.	N.	B.	N.	B.	N.
36	Male—healthy.....	10	18	0	+	0	+	+	++
37	Pregnancy.....	10	16	0	+	+	++	0	++
38	Male—healthy.....	5	16	0	+	++	++	0	++
39	Pregnancy.....	5	16	0	+	0	+	0	+
40	Male—ulcer of stomach.....	10	16	0	+	0	+	++	++
41	Pregnancy.....	10	16	0	+	+	+	+	+
42	Male—healthy.....	10	16	0	++	0	++	0	++
43	Pregnancy.....	10	20	0	+	++	++	++	++
44	Pregnancy.....	10	20	0	+	++	++	++	++
45	Male—healthy.....	10	20	0	+	0	+	+	+
46	Pregnancy.....	10	16	0	+	+	+	0	++
47	Pregnancy.....	10	16	0	+	0	+	0	+
48	Pregnancy.....	5	16	0	+	+	+	0	+
49	Pregnancy.....	10	18	0	++	+	+	+	++
50	Pregnancy.....	10	18	0	++	+	+	+	++
51	Pregnancy.....	10	18	0	+	0	+	++	++
52	Male—healthy.....	10	16	0	+	0	+	0	++
53	Male—healthy.....	10	16	0	+	0	+	0	++
54	Pregnancy.....	10	16	0	+	++	++	0	++
55	Pregnancy.....	10	16	0	0	++	++	0	++
56	Female — general cancer, not pregnant.....	10	20	0	++	0	++	+	++
57	Ca. uterus; not pregnant.....	10	20	0	+	+	+	++	++
58	Male—healthy.....	10	18	0	+	+	+	++	++
59	Pregnancy.....	10	18	0	++	++	++	++	++
60	Pregnancy.....	10	18	0	+	+	++	+	++
61	Male—Ca.	10	16	0	+	0	+	+	+
62	Male—Ca.	10	16	0	++	0	+	0	++
63	Male—Ca.	5	18	0	++	+	++	++	++
64	Male—Ca.	5	18	0	+	0	++	++	++
65	Male—Ca.	5	18	0	+	+	++	++	++
66	Male—Ca.	10	18	0	++	0	+	++	++
67	Male—Ca.	10	18	0	+	0	+	+	++

* In addition to the symbols explained in the footnote to Table 1, in this and the following tables Ca. signifies carcinoma; (1), before incubation; (2), after incubation.

dried placenta (or dried carcinoma) were thoroughly mixed by vigorous shaking and toluene added to cover the mixture completely. The toluene was used to restrict bacterial growth and to prevent drying. These mixtures were incubated at 37.5 C. for twelve hours, and then one-half was drawn off, filtered and tested by the biuret reaction. The remainder was incubated for from four to twelve hours longer and again tested. The best results were obtained by the longer incubation (Table 1). Control tests were carried out with urine alone and with substrate in distilled water. The control urines were always positive to the ninhydrin reagent. All green and blue in the biuret test were considered negative. A positive test varied from purple to lilac.

TABLE 3.—Pregnancy Urines Incubated with Placental and Cancer Substrates

Specimen Number	C.c. Each Test	Hours Incubation	Control (1) Urine		Urine and Placenta		Urine and Carcinoma		Control (2) Urine	
			B.	N.	B.	N.	B.	N.	B.	N.
41	10	16	0	+	+	+	0	+	+	+
43	10	20	+	++	++	++	+	++	++	++
44	10	20	0	+	++	++	0	++	++	++
46	10	16	0	+	+	+	0	+	0	++
47	10	16	0	+	0	+	+	++	0	+
48	5	16	0	+	+	+	0	+	0	+
49	10	18	0	++	+	+	0	+	+	++
50	10	18	0	++	+	+	+	+	+	++
51	10	18	0	+	0	+	0	+	++	++
54	10	16	0	+	++	++	0	+	0	++

From Table 1 it is evident that the minimal period of incubation should not be less than twelve hours, which is considerably longer than noted by Kiutsi. He obtained his results in two hours. It was noteworthy that within twenty-four hours all the specimens except one became positive. The ninhydrin reaction became more positive as the period of incubation increased.

Forty-one pregnancy urines were incubated with placenta, as noted in Table 2, and only twenty-four, or 58.9 per cent., gave a positive reaction and seventeen, or 41 per cent., were negative. Of twenty-six specimens of urine from nonpregnant women examined with placenta, six, or 23 per cent., were positive and twenty, or 77 per cent., were negative. The negative results appeared to be more important than the positive findings.

The next step was to determine the specificity of the test by incubating pregnancy urines with placental and cancer substrates and then treating cancer urines the same way.

Ten pregnancy urines were incubated with cancer tissue and three gave a positive result, while seven gave a negative result. This again speaks for a greater value of negative results than of positive reports.

In malignancy with cancer substrate ten urines were used. These cases were positively carcinomatous, as shown by pathologic reports. In this study four were positive and six were negative. These were incubated at the same time with placental tissue and three, or 30 per cent., gave a positive reaction, while seven, or 70 per cent., gave a negative result.

TABLE 4.—CANCER URINES INCUBATED WITH PLACENTAL AND CANCER SUBSTRATES

Specimen No.	C.c. Each Test	Hours Incubation	Control (1) Urines		Urine and Placenta		Urine and Carcinoma		Control (2) Urine		Diagnosis
			B.	N.	B.	N.	B.	N.	B.	N.	
55	5	18	0	+	0	+	0	+	+	++	Ca. breast
56	10	20	0	++	0	++	+	+	+	++	General Ca.—woman, not pregnant
57	10	20	0	+	+	+	+	++	++	++	Ca. uterus, not pregnant
61	10	16	0	+	0	+	0	+	+	++	Ca. stomach—male
62	10	16	0	++	0	+	0	++	0	++	Ca. stomach—male
63	5	18	0	++	+	++	+	++	++	++	Ca. pancreas—male
64	5	18	0	+	0	++	+	+	++	++	Ca. stomach—male
65	5	18	0	+	+	++	0	+	++	++	Ca. stomach—male
66	10	18	0	++	0	+	0	+	++	++	Ca. breast
67	10	18	0	+	0	+	0	+	+	++	Ca. stomach—male

Of interest was the observation that pregnancy urines reacted with cancer tissue the same way that the cancer urines reacted with the placental substrate.

The biuret reaction was always negative before incubation in both the test and control urines. The reactions after incubation in the control urines gave the following conflicting results:

Total number control urines (nonpregnant patients) (including cancer urines) 26
Positive biuret reactions after incubation 17
Negative biuret reactions after incubation 9

Total number pregnancy urine controls 41
Positive control reactions after incubation 23
Negative control reactions after incubation 18

The results so far obtained in these studies do not coincide with conclusions drawn by the originator of the test. We are inclined to believe that negative results alone should be used as corroborative diagnosis.

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THE RELATION OF PREGNANCY AND CHILDBIRTH TO PELLAGRA IN WOMEN*

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NEW YORK

INTRODUCTION

The exceptionally high prevalence of pellagra among women between the ages of 20 and 45 years has been pointed out in previous publications of this commission and is now generally recognized as characteristic of pellagra in the general population of the southern portion of the United States. In our First Progress Report[1] attention was directed to the observation that pregnancy seems to inhibit the development of pellagrous symptoms, and to the indication that childbirth[2] seems to predispose to an attack of the disease. In the present paper we wish to examine the correlation between the attack of pellagra, either initial or recurrent, on the one hand, and the existence of pregnancy and the event of childbirth on the other, in the histories of all the pellagrins in our series for whom there are definite records in regard to these phenomena.

INITIAL ATTACK OF PELLAGRA DURING PREGNANCY

In the histories of 624 women of childbearing age who suffered their first attack of pellagra in a year prior to 1915 there are twenty definite records of onset of pellagra during pregnancy. For the 101 colored women counted in this group there are no such instances recorded. Excluding the 101 colored women from the present consideration, we find that for twenty, or 3.8 per cent., of the remaining

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* This paper has been written since Dr. Garrison and Dr. Siler were recalled to active duty in the Medical Corps, U. S. Navy, and the Medical Corps, U. S. Army, respectively. They are not personally responsible for the detailed compilation of data or for the specific deductions drawn from them.

1. Siler, J. F., and Garrison, P. E.: An Intensive Study of the Epidemiology of Pellagra, Am. Jour. Med. Sc., 1913, **146**, 42, 238; First Progress Report of the Thompson-McFadden Pellagra Commission, 1914, p. 17.

2. Siler, J. F.; Garrison, P. E., and MacNeal, W. J.: Pellagra, a Summary of the First Progress Report of the Thompson-McFadden Pellagra Commission, Jour. Am. Med. Assn., 1914, **62**, 8; First Progress Report, 1914, p. 3.

523 white women there is history of onset of pellagra during pregnancy. Brief notes for each of these cases follow.

Pellagrin 186, aged 22 at onset of pellagra in March, 1908, was in the second month of pregnancy when she suffered an initial attack of pellagra, manifested by scaling of the hands, stomatitis and diarrhea. The eruption subsided in about two weeks and the attack was evidently a mild one. During the following two years this patient remained entirely free from the disease, again becoming pregnant in August, 1909, and giving birth to a child in May, 1910. In April, 1911, and again in 1912 she suffered mild recurrences of pellagra, but remained free from symptoms of the disease in 1913 and 1914.

Pellagrin 277, aged 25 at the time of onset, first developed a pellagrous erythema in April, 1908, three months before the birth of her child. The attack was evidently mild, although complete, showing erythema of hands, sore mouth, dysentery and nervous symptoms. The eruption remained for about one week, and by winter there was a complete subsidence of all symptoms. In April, 1909, she again had a mild, though complete, attack. There was a very severe recurrence in March, 1910, during which the patient was bedridden for six weeks. In the following two years mild recurrences appeared in April and June, respectively. During 1913 and 1914 this patient remained entirely free from symptoms of pellagra.

Pellagrin 644, aged 26 at onset, was in the fourth month of pregnancy when pellagra first developed in April, 1909. The attack was mild, consisting of sore mouth, followed by an erythema, which subsided in two months. During the following three years she remained free from the disease, being pregnant through the entire pellagra season in 1911. In 1913 she suffered her first recurrence and it was a mild one. In 1914 she remained free from symptoms of pellagra.

Pellagrin 827, aged 33 at onset, was in the seventh month of pregnancy when pellagrous symptoms first developed in August, 1909. The attack was undoubtedly a severe one, for after the birth of her child in October, 1909, the patient was admitted to a hospital, where she died six weeks later.

Pellagrin 95, aged 26 at onset, was in the third or fourth month of pregnancy when she suffered an initial attack of pellagra about May, 1910. The attack was manifested merely by characteristic erythema on the hands. In May, 1911, the patient suffered a mild recurrence, with sore mouth and an erythema on the hands and arms, which subsided in about one week. During the winter of 1911 and also in 1912 she had some mental derangement, but had shown no further recurrence of erythema to July, 1912, the date of the last observation.

Pellagrin 18, aged 29 at the time of onset, developed an initial attack of pellagra in June, 1910, before the birth of her child, the exact date of delivery being unrecorded. The attack was mild, manifested by erythema on the forearms, sore mouth and dysentery, all of which symptoms subsided by July, 1910. In May, 1911, she suffered a severe recurrence with skin, gastro-intestinal and nervous symptoms, which persisted until the following September. Again in April, 1912, this patient had a complete recurrence of pellagra, which had no definite subsidence that year, but ended in death in January, 1913.

Pellagrin 62, aged 21 at onset, was in the fourth month of pregnancy when she first developed an erythema in July, 1911. The attack seems to have been rather severe, with sore mouth, an intermittent diarrhea and an eruption which lasted through most of the summer. In this case there was history of ill health following an operation for pelvic disease in 1909. Early in January, 1912, the patient gave birth to a child and in June, 1912, she suffered a mild recurrence, having been troubled all the spring with diarrhea and insomnia. In the summer of 1913 she had a pronounced attack while pregnant, giving birth to a child in October. In 1914 she is recorded as having recurrences of erythema

in February, May and August, suffering also from sore mouth, insomnia, constipation and loss of appetite during the attack in May.

Pellagrin 568, aged 23 at the time of onset, was in the ninth month of pregnancy when an initial erythema appeared in June, 1911. The patient suffered also from diarrhea, but within two months after the birth of the child the symptoms subsided. After the first attack in 1911 this patient remained quite free from the disease up to the date of our last observation, July, 1914.

Pellagrin 724, aged 24 at onset, suffered her first attack of pellagra in the fall of 1911, when in the first month of pregnancy. The attack consisted merely of the eruption on the hands, which subsided before winter. In June, 1912, after the birth of her child, the patient suffered a recurrence of erythema. She is without record for 1913, but is reported to have suffered a recurrence of erythema in the spring of 1914.

Pellagrin 280, aged 19 at the time of onset, became pregnant the first week in July, 1912, and suffered her initial attack of pellagra in that same month. The only symptom in this first attack was the erythema, which appeared on her hands and subsided by fall. In July, 1913, just three months after childbirth, she suffered a recurrence of pellagra, with erythema, stomatitis, diarrhea and sore tongue. In May, 1914, she had a recurrence of the skin symptoms and sore mouth.

Pellagrin 260, aged 22 at onset, was in the sixth month of pregnancy, when a pellagrous erythema first developed in April, 1912. Her child was born in August, 1912. In this case there were repeated exacerbations of the cutaneous eruption and persistent weakness throughout the fall and winter. She became worse in the summer of 1913. She was admitted to the hospital in Spartanburg and died there Sept. 7, 1913.

Pellagrin 531, aged 29 at the time of onset, was in the second month of pregnancy when she developed an initial attack of pellagra in August, 1912. At this time she had merely a slight erythema. After the birth of her child in March, 1913, she had a recurrence, beginning in May, manifested by erythema, sore tongue and dysentery. In 1914 she reported better health than she had had for years, and she remained free from symptoms during that year.

Pellagrin 701, aged 31 at onset, was in the eighth month of pregnancy when an erythema first appeared on her hands in March, 1912. The attack was a very slight one, evidenced merely by the pellagrous eruption. In the spring of 1913 she had a severe recurrence. In May, 1914, she suffered a recurrence of the skin eruption.

Pellagrin 253, aged 35 at onset, was in the eighth month of pregnancy when a pellagrous erythema first developed in May, 1912. The eruption, which was the only symptom in this initial attack, extended over the hands and forearms and subsided by August, 1912. The patient remained quite free from recurrence of the disease in 1913 and 1914.

Pellagrin 1167, aged 21 at onset, suffered an initial attack of pellagra in July, 1913, just one month before the birth of a child. The only symptom in this first attack was the typical erythema, which cleared up after her child was born. In May, 1914, she had a recurrence of the erythema.

Pellagrin 977, aged 22 at the time of onset, was in the eighth month of pregnancy when the initial erythema appeared in July, 1914. The symptoms consisted of eruption on the forearms and hands, with sore mouth and indigestion.

Pellagrin 1267, aged 28 at the time of onset, suffered her initial attack of pellagra a few days before the birth of her child in January, 1914. This attack was manifested by gastro-intestinal as well as skin symptoms, and these did not disappear until some time after the birth of the child.

Pellagrin 958, aged 29 at onset, had suffered indigestion for two months prior to the development of pellagra in February, 1914, three months before the birth of her child. In this case there was an early subsidence of skin symptoms, but diarrhea persisted to the date of last observation in July, 1914.

Pellagrin 1078, aged 42 at the time of onset, was well advanced in pregnancy

when pellagra first developed in the latter part of July, 1914. With the exception of the erythema, which recurred that summer, and sore mouth, the patient's health was not markedly impaired.

Pellagrin 964, age not recorded, suffered a first attack of pellagra in January, 1914, just before the birth of her child. The onset was mild, but in the following June the erythema recurred and with it stomatitis and marked general weakness.

TABLE 1.—SUMMARY OF THE TWENTY INITIAL ATTACKS OF PELLAGRA WHICH OCCURRED DURING PREGNANCY

Pellagrin Number	Age at Onset	Date of Onset	Month of Pregnancy	Character of Attack	History During Pregnancy
186	22	March, 1908	Second	Mild	Recovery
277	25	April, 1908	Sixth	Mild	Recovery
644	26	April, 1909	Fourth	Mild	Recovery
827	33	August, 1909	Seventh	Severe	Persisted*
95	26	May, 1910	Third or Fourth	Mild	Recovery
18	29	June, 1910	Middle	Mild	Recovery
62	21	July, 1911	Fourth	Severe	Recovery
508	23	June, 1911	Ninth	Mild	Recovery
724	24	Fall, 1911	First	Mild	Recovery
280	19	July, 1912	First	Mild	Recovery
260	22	April, 1912	Sixth	Severe	Persisted†
531	29	August, 1912	Second	Mild	Recovery
701	31	March, 1912	Eighth	Slight	Recovery
253	35	May, 1912	Eighth	Mild	Recovery
1167	21	July, 1913	Eighth	Mild	Recovery
977	22	July, 1914	Eighth	Mild	Recovery
1207	28	January, 1914	Ninth	Mild	Recovery
958	29	February, 1914	Sixth	Mild	Recovery
1078	42	July, 1914	"Advanced"	Mild	Recovery
964	Not recorded	January, 1914	Ninth	Mild	Recovery

* This patient died of pellagra six weeks after childbirth in the fall of 1909.
† This patient survived the year, giving birth to her child in August, 1912. She did not regain her health, but continued to suffer from pellagra until death in September, 1913.

Among the 523 recorded initial attacks of pellagra in white women in the age period 12 to 49 years there are only twenty, or 3.8 per cent., in which the onset occurred during pregnancy. None of these twenty died of pellagra during pregnancy. In fact, with a single exception, those who developed the disease early in pregnancy suffered only mild attacks. This exceptional case, Pellagrin 62, had been in ill health following a pelvic operation in 1909 and she suffered a rather severe initial attack of pellagra in July, 1911, in the fourth month of pregnancy. In one instance, Pellagrin 827, the attack appeared in the seventh month of pregnancy, persisted until childbirth and ended in

death six weeks later. In another case, Pellagrin 260, pellagra appeared in the sixth month of pregnancy and persisted until long after delivery, the patient dying of pellagra in the following year. The comparative infrequency[3] of onset of pellagra during pregnancy and the benign character of the attack when it does occur indicate that during pregnancy there is an increased resistance to pellagra.

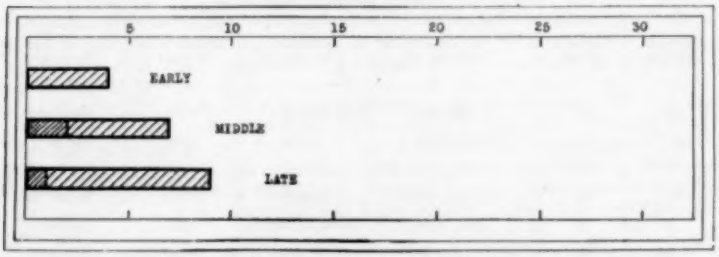

Fig. 1.—Initial attacks of pellagra which began during the first, second and last three months of pregnancy, respectively. The total length of the bar indicates the number of instances of onset in the respective three-month period. The more thickly shaded portion indicates the number of instances in which the attack of pellagra was severe.

THE RELATION OF CHILDBIRTH TO INITIAL ATTACK

Frequently in the histories of pellagrous women childbirth just previous to the initial attack is recorded as a possible predisposing factor. On closer examination of the records of these mothers the rather large number of instances in which a childbirth in the early part of the year, usually from January to April, has been followed within a few months, or even weeks, by an initial development of pellagrous symptoms invites consideration. In the records of the 624 pellagrous women of childbearing age (12 to 49 years) in our series to the end of 1914 there are fifty-seven who gave a history of childbirth within six months preceding onset. This group of 624 women includes 101 colored pellagrins. Because of the comparatively small number of records for the colored race and doubtless, also, because of the incompleteness of the records, there are recorded but four cases of possible predisposing childbirth, or 4 per cent., for the colored race. After subtracting from the total group of women the 101 colored pellagrins,

3. The condition of pregnancy exists at any given time in more than 3.8 per cent. of the women between 12 and 49 years of age in this population. If only 3.8 per cent. of them were pregnant at a given time on the average, then the average woman would be pregnant 3.8 per cent. of 38 years (12 to 49, inclusive), or 1.44 years, about seventeen months, and would, therefore, fail to bring forth more than two children during her childbearing period, a number too small to maintain the population and unquestionably far below the actual average number in this population.

we find that fifty-three, or 10.1 per cent., of the remaining 523 white women suffered onset of pellagra following a childbirth.

If the total birth records in Spartanburg County for a given year were available for comparison with the total number of initial attacks of pellagra among mothers delivered in the respective year, it would be highly interesting to ascertain in what ratio women who had recently borne a child were attacked by this disease. We have, however, the histories of the 523 white women who did suffer attack. The fact that a very large proportion of all women in these southern mill villages do bear children and that standards of living and environmental influences are generally similar reduces them to a fairly uniform basis for comparison.

TABLE 2.—COMPARISON OF THE NUMBER OF WOMEN FROM 12 TO 49 YEARS OF AGE, WITH ONSET OF PELLAGRA IN EACH YEAR, WITH THE NUMBER HAVING RECORD OF CHILDBIRTH JUST PRIOR TO ONSET OF THE DISEASE

Year of Onset	White Women			Colored Women		
	Total Initial Attacks	Instances of Childbirth Just Prior to Onset		Total Initial Attacks	Instances of Childbirth Just Prior to Onset	
		Number	Per Cent.		Number	Per Cent.
Before 1908	28	3	10.7	4	0	0.0
1908	13	1	7.5	1	0	0.0
1909	30	1	3.4	2	0	0.0
1910	70	7	10.0	12	0	0.0
1911	117	9	7.7	14	0	0.0
1912	80	5	6.3	20	0	0.0
1913	114	20	17.5	23	1	4.3
1914	71	7	9.9	25	3	12.0
Total	523	53	10.1	101	4	4.0

In Table 2 are summarized the total number of incident cases of pellagra among women in the age period 12 to 49 years at the time of onset of pellagra, together with the total recorded cases of childbirth prior to onset among these women. These are grouped according to race and year of onset. That 10.1 per cent. of the pellagrous white women suffered onset of pellagra following childbirth suggests very strikingly that the puerperal state may predispose to pellagra.[4]

4. Less than 10 per cent. of the women in this population, on the average, will have borne a child within six months. If 10 per cent. of them should have borne a child within six months, at any given time, it would require that the average woman bring forth one child every five years, or a little more than seven children in the thirty-eight years (12 to 49, inclusive) of her assumed childbearing period. This figure is unquestionably too large for the average woman of this population.

This hypothesis may be strengthened by a detailed examination of the histories of these mothers. In the citations which follow there have been recorded, so far as the records permit, any complicating disorders which might have influence on the physical condition of the mother prior to onset of pellagra. The cases are grouped for consideration according to the year of onset, with the exception of those incident in a year prior to 1908. The latter are considered in one group. Of these, twenty-eight were of childbearing age at time of onset and for three of them there is history of childbirth within a period of six months preceding the onset of pellagra.

Pellagrin 354 was 16 years old when she suffered her first attack of pellagra in the summer of 1901, following childbirth. In this case is recorded the complication of puerperal insanity, which appeared just prior to development of pellagra. The initial attack was a severe one, in which skin, gastro-intestinal and mental symptoms were well marked. The patient gave a history of recurrent erythema and mental disturbance nearly every summer to death in February, 1910.

Pellagrin 1024, aged 23 at onset, had her initial attack in the summer of 1905, following a childbirth. For this case there is a history of freedom from attack for four years subsequent. But in 1910 she again developed frank symptoms and continued to suffer recurrences each summer to 1914, inclusive.

Pellagrin 543, aged 36 at onset, suffered her first attack of pellagra in the spring of 1906, about two months after childbirth. She suffered annual recurrence to 1913. In 1914 she remained free from recurrence up to the time of her last observation, which was made in July.

Of the thirteen white women of childbearing age with onset of pellagra in 1908, there is one for whom there is recorded a childbirth preceding the onset of pellagra.

Pellagrin 1024, aged 23 at onset, had her initial attack in the summer of 1905, 1908. In July of the same year symptoms of pellagra first developed and persisted to her death in January, 1909.

In 1909 there were thirty instances of initial attack of pellagra among white women of childbearing age. For one of these there is a history of childbirth prior to onset.

Pellagrin 59, aged 35 at onset, first developed symptoms of pellagra in March, 1909, three months after the birth of her child. At this time she suffered from loss of appetite, insomnia and nervousness. The erythema in this first attack did not appear until June. She suffered a recurrence each summer thereafter to 1914, during which year she remained free from symptoms.

Of the seventy white women of childbearing age who suffered initial attacks in 1910, there were seven who gave a history of childbirth prior to attack of pellagra.

Pellagrin 702, aged 23 at onset, suffered her first attack in October, 1910. After the birth of twins earlier in the year menstruation had not reappeared. She suffered recurrences in 1911 and 1912. In 1913, after a childbirth in April, she had a recurrence of symptoms in May. In April, 1914, she also suffered recurrence.

Pellagrin 544, aged 23 at the time of onset, first developed pellagra in the fall of 1910, about one month after the birth of her child. In 1911 she gave birth to a child in the summer and escaped recurrence of symptoms during that year and also during 1912; but a few days after childbirth in April, 1913, she suffered a recurrence. There is no record for 1914.

Pellagrin 112, aged 24 at onset, gave birth to a child in March, 1910. The following June she suffered her first attack of pellagra. In 1911 she had a recurrence, but escaped in 1912. In April, 1913, while she was pregnant, she suffered a recurrence. In 1914 she remained free from symptoms.

Pellagrin 998, aged 27 at the time of onset, had her first attack of pellagra in June, 1910, just one month after the birth of a child. This patient gave a history of indigestion for several months before the birth of her child and of sore mouth, persistent diarrhea and insomnia prior to the development of the erythema. Her ill health continued till death in June, 1911.

Pellagrin 860, aged 32 at onset, first developed an erythema in August, 1910, shortly after giving birth to a child. During 1911, 1912 and 1913 she remained quite free from symptoms of pellagra. In 1914, however, while she was again pregnant, she suffered a recurrence in February, about five weeks before her child was born. The pellagrous symptoms persisted and the patient died in June, 1914.

Pellagrin 292, aged 39 at onset, gave birth to a child in January, 1910. She had a definite attack of pellagra in the following June. She suffered recurrences each year to 1914, during which year she is said to have remained free from symptoms.

Among the 117 white women of childbearing age incident in 1911, there are nine instances of onset following a childbirth.

Pellagrin 269, aged 23 at onset, suffered her initial attack in May, 1911, just three weeks after the birth of her child. She had recurrences each year thereafter to 1914, inclusive. In 1913, while she was pregnant, pellagrous symptoms developed in May, two months before the birth of her child.

Pellagrin 980, aged 25 at onset, gave birth to a child in June, 1911. At that time she was suffering from severe stomatitis and the erythema appeared soon after. The mental symptoms became marked in this case, and after she had made an attempt to take the life of her child, a nurse was in constant attendance. The pellagrous symptoms recurred in 1912 and persisted until death in August of the same year.

Pellagrin 678, aged 26 at onset, gave birth to a child in March, 1911. In the following summer symptoms of pellagra first developed. In 1912 she was pregnant during the pellagra season, giving birth to a child in October. She remained quite free from symptoms during that year and also during 1913 and 1914.

Pellagrin 122, aged 27 at time of onset, suffered an initial attack of pellagra two or three weeks after the birth of her child in March, 1911. She had no recurrence of symptoms to 1914, inclusive. In 1912 she was pregnant through the greater part of the pellagra season, giving birth to a child in August.

Pellagrin 81, aged 27 at onset, gave birth to a child in May, 1911. She suffered from diarrhea for a month before the baby was born, and the erythema appeared in June. In 1912 she had a recurrence of erythema. She remained quite free from symptoms during 1913 and 1914.

Pellagrin 883, aged 30 at the time of onset, first developed an erythema five weeks after the birth of her child in March, 1911. She remained free from symptoms during 1912, but suffered a recurrence in 1913. In 1914 she escaped recurrence.

Pellagrin 281, aged 32 at onset, gave birth to a child in February, 1911. Her baby had persistent bowel trouble and died at the age of 4 months. In July, 1911, shortly after the death of the child, the mother had her initial attack of pellagra. She remained free from symptoms of the disease in 1912, during which

year she was pregnant until November. She also escaped recurrence in 1913, but suffered an attack in 1914.

Pellagrin 1137, aged 34 at onset of pellagra in the spring of 1911, gives a history of the appearance of pellagrous symptoms following the birth of her two children in 1911 and 1913, respectively. In 1912 and 1914 she remained free from the disease.

Pellagrin 238, aged 38 at onset, gave birth to a child in February, 1911, and suffered her first attack of pellagra in the following May or June. After that she remained entirely free from symptoms up to the last observation in June, 1914.

Of the eighty white women of childbearing age with onset in 1912, there are five with definite record of childbirth just prior to the initial attack of pellagra.

Pellagrin 596, aged 19 at onset, gave birth to a child in December, 1911. The following spring (1912) she had her initial attack of pellagra. In July, 1913, she had a recurrence. She was pregnant in 1914 from February and remained free from symptoms during that year.

Pellagrin 614, aged 27 at time of onset, suffered her initial attack of pellagra shortly after giving birth to a child in August, 1912. In 1913 and 1914 she suffered complete, though mild, recurrences in May and March, respectively. In 1914 she was pregnant until July.

Pellagrin 1000, aged 29 at onset, was in poor health after the birth of her last child in January, 1912. The following March (1912) the erythema appeared for the first time. In March, 1913, she suffered a recurrence of symptoms and she died in April, 1913.

Pellagrin 164, aged 30 at the time of onset, suffered an initial attack of pellagra in June, 1912, two months after the birth of a child. In 1913 she was pregnant from June. She remained free from symptoms during 1913. In February, 1914, she gave birth to a child, but up to June of that year had suffered no recurrence.

Pellagrin 1185, aged 35 at onset, gave birth to a child in January, 1912. She had her initial attack in the following April (1912). In 1913 she remained free from symptoms, but suffered a recurrence in 1914.

In 1913 there were 114 white women of childbearing age who had an initial attack of pellagra. For twenty-one of these there is a record of a childbirth prior to onset.

Pellagrin 798, aged 18 at time of incidence in June, 1913, had given birth to a child three months before. She died of pellagra in July, 1913.

Pellagrin 635, aged 20 at onset, had given birth to a child late in December, 1912. Pellagra developed the following April (1913). During 1914 she remained free from symptoms.

Pellagrin 872, aged 22 at the time of onset, gave birth to a child in May, 1913. The following June she developed an initial erythema. In 1914 she remained free from symptoms.

Pellagrin 549 was 23 years old when she had her first attack of pellagra in June, 1913. She had given birth to a child the preceding March. She remained free from recurrence the following year.

Pellagrin 1034, aged 23 at onset, suffered a first attack of pellagra in August, 1913, just one month after giving birth to a child. She had a recurrence in July, 1914.

Pellagrin 837, aged 23 at the time of onset, gave birth to a child in March, 1913. She developed pellagrous symptoms for the first time in August, 1913. In 1914 she escaped recurrence.

Pellagrin 741, aged 24 at onset, suffered her initial attack of pellagra in June, 1913, two months after a childbirth. She had a severe recurrence in May, 1914.

Pellagrin 639, aged 24 at onset, gave birth to a child in February, 1913. The following May (1913) she had her first attack of pellagra. In 1914 she was reported to have had a severe recurrence during the spring and summer.

Pellagrin 552, aged 24 at onset, gave birth to a child in January, 1913. The following May (1913) she developed pellagra for the first time. In 1914 she remained free from symptoms.

Pellagrin 559, aged 24 at onset, gave birth to a child in February, 1913. She suffered her initial attack the following May. In 1914 she escaped recurrence.

Pellagrin 904, aged 25 at onset, gave birth to a child in September, 1913. Her health was very poor after childbirth, and in December, 1913, she developed symptoms of pellagra. She suffered a recurrence of the disease in 1914.

Pellagrin 576, aged 25 at onset, gave birth to a child in December, 1912. She had poor health during the months following, and in June, 1913, suffered her first attack of pellagra. In 1914 she had a slight recurrence of symptoms.

Pellagrin 591, aged 26 at onset, was nursing her 5-months-old baby when pellagra first developed in June, 1913. She is without record for 1914.

Pellagrin 458, aged 26 at onset, gave birth to a child in January, 1913. She began to feel ill in March, but continued to nurse her baby until pellagra developed in May, 1913. She remained free from symptoms during 1914.

Pellagrin 498, aged 28 at onset, suffered her first attack in June, 1913, about three months after the birth of her child. During 1914 she was pregnant until September. She remained free from symptoms of pellagra in 1914.

Pellagrin 700, aged 29 at onset, gave birth to a child in May, 1913, and pellagra developed at about the same time. She grew progressively worse and was admitted to the hospital on August 10, where she died two weeks later.

Pellagrin 1031, aged 29 at onset, had suffered from stomatitis in the fall of 1912. She gave birth to a child in January, 1913, and diarrhea developed shortly afterward, followed by a severe erythema in July. These symptoms persisted until death in October, 1913.

Pellagrin 537, aged 29 at onset, was in poor health before and following the birth of her child in October, 1912. In March, 1913, she developed an erythema for the first time. During 1914 she was pregnant until September, and remained free from symptoms during that year.

Pellagrin 665, aged 30 at onset, had an initial attack in May, 1913. Following the birth of her child three months before, she had been in poor health. During 1914 she remained free from symptoms.

Pellagrin 610, aged 32 at the time of onset, gave birth to a child in December, 1912. In the following June (1913) she suffered her initial attack of pellagra. In 1914 she escaped recurrence of symptoms.

Pellagrin 294, aged 36 at onset, gave birth to a child in February, 1913, and had her first attack of pellagra in April. She grew worse and was admitted to the Good Samaritan Hospital in July. Against the advice of her physician she left the hospital in August and died in October, 1913.

In 1914 there were seventy-one incident cases of pellagra recorded among white women of childbearing age. For seven of the white women, or 9.9 per cent., there is record of a possibly predisposing childbirth.

Pellagrin 1250, aged 20, had a severe attack of dysentery in May, 1914, about two weeks after the birth of her child. The pellagrous erythema did not appear until the second week in October.

Pellagrin 922, aged 22 at onset, gave birth to a child the last of February, 1914. She developed initial symptoms of pellagra the last of April.

Pellagrin 1155, aged 22 at time of onset, gave birth to a child in the latter part of January, 1914. She first developed erythema in the following July.

Pellagrin 954, aged 26 at onset, suffered an initial attack in June, 1914, about six weeks after a childbirth.

Pellagrin 1019, aged 27, gave birth to a child early in May, 1914, and first developed pellagrous symptoms about two weeks after delivery.

Pellagrin 1011, aged 29 at onset, gave birth to a child the last of May, 1914, and two months later suffered an initial attack of pellagra. In the case of this patient there was a complication of diarrhea which had persisted from the fall of 1913.

Pellagrin 1161, aged 33 at time of onset, gave birth to a child in March, 1914, and developed initial pellagrous symptoms about four weeks after delivery.

TABLE 3.—PELLAGRINS WHO SUFFERED THE INITIAL ATTACK WITHIN ONE MONTH AFTER CHILDBIRTH

Pellagrin Number	Age at Onset	Date of Childbirth	Onset of Pellagra	Interval Indicated by the Original Record
White Women—				
354	16	Summer, 1901	Summer, 1901	Following childbirth
1024	23	Summer, 1905	Summer, 1905	Following childbirth
1107	18	Summer, 1908	July, 1908	Following soon after
544	23	Fall, 1910	Fall, 1910	About one month after childbirth
998	27	May, 1910	June, 1910	One month
860	32	August, 1910	August, 1910	Following childbirth
269	23	May, 1911	May, 1911	Three weeks after childbirth
980	25	June 5, 1911	June, 1911	Soon after childbirth
122	27	March, 1911	Late March, 1911	Two or three weeks
81	27	May 8, 1911	June, 1911	About one month
1137	34	Spring, 1911	Spring, 1911	Following childbirth
614	27	August, 1912	September, 1912	Following childbirth
1034	23	July 1, 1913	August, 1913	One month
700	29	May, 1913	May, 1913	At about same time
1019	27	May, 1914	May, 1914	About two weeks
1161	33	March, 1914	April, 1914	Four weeks

Of the 101 colored women incident before 1915, there is a history of a previous childbirth for four.

Pellagrin 410, aged 25 at onset, first developed symptoms of pellagra in the spring of 1913, following a childbirth, the exact date of which is not recorded. She died of pellagra in July, 1913.

Pellagrin 943, aged 21, gave birth to a child in January, 1914. During the early months of her pregnancy she had lived with her grandmother, Pellagrin 539, who died of pellagra during the summer of 1913. The patient remained free from symptoms during that year. In May, 1914, however, four months after the birth of her child, she developed pellagra for the first time.

Pellagrin 1094, aged 21 at onset, gave birth to a child early in June, 1914. In August she suffered an initial attack of pellagra.

Pellagrin 923, aged 24 at the time of onset, developed pellagra for the first time in May, 1914, about four or five months subsequent to a childbirth. The symptoms persisted until death, June 24, 1914.

Of the ninety-six women aged 12 to 49 years with initial attack of pellagra in 1914, there are, therefore, ten instances of a possibly predisposing childbirth, or 10.4 per cent., for both races. If these incident cases with their proportion of prior childbirths are added to those of women incident before 1914 with their respective cases of childbirth, we have a total of fifty-seven possibly predisposing childbirths among 624 pellagrous women, or a percentage of 9.1 for both races. This

TABLE 4.—Pellagrins Who Suffered the Initial Attack Later Than One Month But Within Three Months After Childbirth

Pellagrin Number	Age at Onset	Date of Childbirth	Onset of Pellagra	Interval Indicated by the Original Record
White Women—				
543	36	Early in 1906	Spring, 1906	About two months
702	23	Late summer or fall, 1910	October, 1910	About three months
112	24	March 19, 1910	June, 1910	Three months
883	30	March 21, 1911	April, 1911	Five weeks
1000	29	January, 1912	March, 1912	Two months
164	30	April, 1912	June, 1912	Two months
1185	35	January, 1912	April, 1912	Three months
798	18	March, 1913	June, 1913	Three months
872	22	May 10, 1913	July, 1913	Two months
549	23	March, 1913	June, 1913	Three months
741	24	April, 1913	June, 1913	Two months
639	24	February, 1913	May, 1913	Three months
559	24	February, 1913	May, 1913	Three months
904	25	September, 1913	December, 1913	Three months
498	28	March, 1913	June, 1913	Three months
665	30	February, 1913	May, 1913	Three months
294	36	February, 1913	April, 1913	Two months
922	22	February, 1914	April, 1914	Two months
954	26	May, 1914	June, 1914	Six weeks
1011	29	May, 1914	July, 1914	Two months
Colored Women—				
410	25	Early in 1913	Spring, 1913	Not recorded
1094	21	June, 1914	August, 1914	Two months

indicated percentage is undoubtedly too small, for the records of very many of these 624 women are incomplete, especially in respect to dates of childbirths. Indeed it was not until the year 1913 that the subject of the possible predisposition of puerperal women to pellagra was given any particular attention. Thus, we find the tabulations for that year and for 1914 more complete and, therefore, more reliable criteria in regard to this relationship than are the recorded data for the pre-

ceding years. In this connection it should be mentioned that the records for the latter part of 1914 are incomplete because of the termination of the field work of the commission in the fall of that year.

The fifty-seven examples of onset of pellagra within six months after childbirth are presented in Tables 3, 4 and 5, separately, according to the length of the interval between childbirth and the onset of the initial attack. Those with onset of pellagra in the first month

TABLE 5.—PELLAGRINS WHO SUFFERED THE INITIAL ATTACK LATER THAN THREE MONTHS BUT WITHIN SIX MONTHS AFTER CHILDBIRTH

Pellagrin Number	Age at Onset	Date of Childbirth	Onset of Pellagra	Interval Indicated by the Original Record
White Women—				
59	35	December, 1908	June, 1909	Six months
292	39	January, 1910	June, 1910	Five months
678	26	March, 1911	Summer, 1911	Not recorded
281	32	February, 1911	July, 1911	Five months
238	38	February, 1911	May or June, 1911	Three or four months
506	19	December, 1911	Spring, 1912	Not recorded
635	20	Dec. 22, 1912	April, 1913	Four months
837	23	March, 1913	August, 1913	Five months
502	24	January, 1913	May, 1913	Four months
576	25	December, 1912	June, 1913	Six months
581	26	January, 1913	June, 1913	Five months
428	26	Jan. 10, 1913	May, 1913	Four months
1031	29	January, 1913	July, 1913	Six months
537	29	October, 1912	March, 1913	Five months
610	22	December, 1912	June, 1913	Six months
1250	20	May, 1914	October, 1914	Five months
1155	22	January, 1914	July, 1914	Six months
Colored Women—				
943	21	January, 1914	May, 1914	Four months
923	24	January or February, 1914	May, 1914	Three or four months

after delivery are shown in Table 3. These number sixteen, all white women, and constitute 32.2 per cent. of the white women in the whole group. In Table 4 are shown the twenty-two pellagrins who suffered initial attack in the second and third months following childbirth. Twenty of these were white women, making up 37.7 per cent. of the fifty-three white women in the whole group. In Table 5 are shown nineteen pellagrins who developed the disease in the fourth, fifth or sixth month following childbirth, seventeen of these being white women. This group is approximately equal to that shown in Table 3,

indicating that onset of pellagra has been about three times as common in the first month following pregnancy as in either the fourth, fifth or sixth month following such an event. Furthermore, this group shown in Table 5 is one half as large as the sum of those in Tables 3 and 4, indicating that onset of pellagra has been about twice as frequent in the first three months following childbirth as it has been in the succeeding three months. These relationships certainly suggest that the event of childbirth may be an important factor in predisposing to pellagra and that it may also determine to some degree the onset of definite symptoms of the disease.

Another interesting relation shown by these tables is that of season. Of the sixteen childbirths recorded in Table 3, no less than fifteen occurred in the spring or summer months. On the other hand, of the nineteen childbirths shown in Table 5, only two occurred during spring

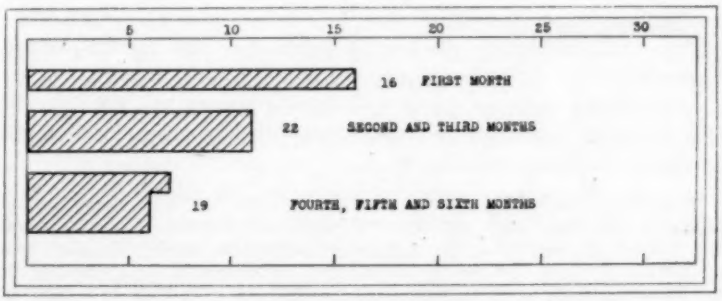

Fig. 2.—The number of initial attacks of pellagra following childbirth. Note that there were sixteen in the first month, twenty-two in the second and third months and nineteen in the fourth, fifth and sixth months after delivery.

or summer and no less than fourteen of them took place in the three winter months, December, January and February. It is evident that the danger of pellagra developing within a few weeks after childbirth is greatest when the pregnancy terminates during the spring or summer and, from what has been said above, it would seem that the cases of this kind are the ones to which childbirth has the most definite relation.

THE RELATION OF RECURRENT ATTACK OF PELLAGRA TO PREGNANCY AND CHILDBIRTH

In the preceding discussion the relation of the first or initial attack of pellagra to certain physiologic conditions of the woman have been considered. In this section we purpose to consider the subsequent behavior of all women who survived the year of initial attack and who presented a definite record of subsequent pregnancy or childbirth,

in order to ascertain whether there is any correlation, either positive or negative, between the pregnant state and recurrence of pellagra or between the puerperal state and recurrence of pellagra. In the preceding paper[5] of this series has been presented the correlation between recurrence of pellagra, on the one hand, and race, sex and age, on the other, and it was there suggested that pregnancy and childbirth seemed to influence the behavior of the women in respect to recurrence. In that paper all the women who suffered the initial attack of pellagra previous to the year 1914 were considered. Here, those of this same group for whom there is a definite record of pregnancy or of childbirth after the onset of pellagra will be considered.

There are, in all, eighty-three white women and four colored women in this category, including fifteen[6] of the twenty women who suffered the initial attack of pellagra during pregnancy. For a considerable part of these, however, there is a definite record of more than one pregnancy per patient, so that the number of instances of pregnancy or of childbirth in surviving pellagrins available for the present discussion is 101 in white women and five in colored women.

Among the white pellagrins with onset previous to 1908 there is only one with a definitely recorded history of pregnancy or childbirth subsequent to onset of pellagra.

Pellagrin 70, aged 27, suffered her initial attack in 1905. Every spring thereafter she had bowel trouble, sore mouth and nervous disturbance, but the evidence of definite recurrent erythema is lacking until 1913. She was pregnant in 1907 or 1908 and miscarried, but the record of this pregnancy is not definite enough to be of value here. In 1913 she gave birth to a child in April, suffered a definite recurrence of pellagra in August and died in January, 1914, of pellagra and complicating disorders.

Of the white pellagrins who survived the initial attack in 1908 there are five with definite record of pregnancy or of childbirth after the onset of pellagra.

For the history of Pellagrin 186 see the section on Initial Attack During Pregnancy.

Pellagrin 432, aged 24, suffered her first attack of pellagra in 1908, with severe recurrence in 1909, but no recurrence in 1910. She became pregnant in August, 1910, giving birth to her child in May, 1911. The years 1911 and 1912 were passed without reappearance of pellagra. She again conceived in August, 1912, and was delivered in May, 1913. This time an attack of pellagra

5. Siler, J. F., Garrison, P. E., and MacNeal, W. J.: The Relation of Recurrent Attacks of Pellagra to Race, Sex and Age of the Patient and to Treatment of the Disease, THE ARCHIVES INT. MED., 1916, **18,** 652.

6. For three cases with onset of pellagra in 1914 during pregnancy, namely, Pellagrins 977, 958 and 1078, data subsequent to delivery are not available. For two cases, Pellagrins 827 and 260, with initial attack during pregnancy, pellagrous symptoms persisted after delivery. These five cases are excluded from this category.

appeared during the puerperium and there was another definite recurrence in 1914.

For history of Pellagrin 277 see the section on Initial Attack During Pregnancy.

Pellagrin 987 was 25 years old in the summer of 1908, when her pellagrous symptoms first appeared. In September, 1908, she became pregnant. She suffered a mild recurrence of pellagra the following spring before childbirth, which occurred June 10, 1909. Every spring thereafter to 1914, inclusive, she had recurrences, mild symptoms developing in 1912 during a pregnancy, which was terminated by childbirth in November, 1912.

Pellagrin 37, aged 32 at the time of onset, suffered her first attack in the fall of 1908. Each spring thereafter she had a recurrence, although in 1911, after an early recurrence, she remained entirely free from symptoms after April, when she became pregnant, being delivered the following December. In April, 1912, while still nursing her baby, she suffered another recurrence, and pellagra again recurred the following spring (1913). In August, 1913, she again became pregnant and remained free from symptoms of pellagra in 1914 until after childbirth on May 15. At our last observation in June, 1914, slight but definite signs of a recurrence of pellagra were already present.

Of the seven instances of pregnancy subsequent to onset of pellagra there are, therefore, five in which the pregnancy terminated in the early summer and two in which childbirth occurred in the late fall. Of the five pregnancies terminating in the summer, four were free from recurrence of pellagrous symptoms, while in one instance a mild attack of pellagra developed in the sixth or seventh month. In the two instances of childbirth in the fall the patient remained quite free from the disease during pregnancy in one and in the other a mild recurrence appeared in the first or second month of pregnancy.

There were two instances of a childbirth following onset during pregnancy. In one of these cases the childbirth occurred in July, in the other it occurred in the fall. There were, therefore, nine instances of childbirth subsequent to onset of pellagra, six occurring in the summer and three in the fall. In seven of these the mother remained entirely free from symptoms for a period of at least three months following her delivery, and in two there was recurrence in the summer within four weeks after the childbirth.

Of the white pellagrins with initial attack in 1909, there are nine for whom definite records of pregnancy, or of childbirth subsequent to onset, are available.

Pellagrin 817, aged 17 at onset in 1909, became pregnant soon after and remained entirely free from any symptoms of pellagra up to our last observation in 1914.

Pellagrin 94 suffered her first attack in April, 1909, when 21 years of age. The following year she had a recurrence in April. In 1911 she suffered a very severe recurrence, being confined to bed for three months. In July, 1911, she became pregnant, giving birth to a child in March, 1912. In this case a pregnancy during the cool season was succeeded by a very mild recurrence in June, 1912. Late in the summer of 1912 she became pregnant and aborted in October, 1912. In February, 1913, she became pregnant again and

remained entirely free from symptoms for that year. In 1914 she again suffered a recurrence of pellagra.

Pellagrin 178 was 23 years old at the time of her initial attack in the spring of 1909. She recovered and remained free from symptoms until 1912. After childbirth early in 1912 she suffered a recurrence of pellagra in March and in 1913 she had another mild recurrence. In January, 1914, she became pregnant and escaped recurrence of pellagra to the date of the last observation, July 7, 1914.

Pellagrin 151 suffered her first attack in March, 1909, when 23 years of age. Each subsequent spring she had a recurrence up to and including 1912. In July, 1912, she became pregnant. Her baby was born in March, 1913. She remained entirely free from symptoms of pellagra during 1913 and 1914.

Pellagrin 119, aged 25, had her initial attack in July, 1909. The following March she became pregnant and remained free from symptoms of pellagra during 1910. In 1911 she had no recurrence and again became pregnant in August. After a winter pregnancy, terminated by childbirth April 29, 1912, she remained quite free from symptoms during 1912, but in 1913 she suffered a recurrence. She is without record in 1914.

Pellagrin 140, aged 25, suffered her first attack in May, 1909. She had a recurrence in 1910, and in 1911 the symptoms reappeared in April. In June, 1911, she became pregnant. After giving birth to a child in February, 1912, she had a very severe recurrence in June, 1912, and died of pellagra on Aug. 17, 1912.

Pellagrin 80 was 26 years old when she suffered her initial attack early in the summer of 1909. In 1910 she had no recurrence of pellagra, but in July, 1911, while she was pregnant, she had a very severe recurrence and after delivery in August, 1912, her hands broke out a second time. Thereafter, from 1912 to 1914, inclusive, she was entirely free from symptoms of the disease.

For history of Pellagrin 644 see section on Initial Attack During Pregnancy.

Pellagrin 1346, aged 30 at the time of onset of pellagra in 1909, suffered recurrences in 1910 and 1911. In 1912 she was pregnant until September and during that year remained free from symptoms. In 1913 she also escaped recurrence of the erythema, although she had sore mouth and some physical weakness. She is without record for 1914.

Among the white pellagrins with initial attack in 1909 there were, therefore, thirteen instances of pregnancy subsequent to onset of pellagra. In five cases childbirth occurred in the early months of the year (from January to April, inclusive), in one case it occurred in late summer and in six instances the period of pregnancy extended over the entire pellagra season, with termination in the fall. In one instance the exact season of gestation was not recorded. With one exception the patients were entirely free from symptoms of pellagra during all these pregnancies. In the case of the pregnancy with summer termination, however, a severe recurrence was suffered in the eighth month and after childbirth in August a second erythema developed within four weeks.

There was one case of initial attack during pregnancy with subsequent childbirth, making a total of fourteen instances in this category in 1909. For one 1914 pregnancy records of subsequent history are not available. For ten of the thirteen remaining cases there was no recurrence of pellagra for three months subsequent to delivery; two

of the instances of childbirth early in the year were followed by recurrence within three months and the one instance of childbirth late in the summer was followed by a recurrence of pellagra within one month.

Among the white pellagrins with initial attack in 1910 there are seventeen with a definite history of pregnancy or of childbirth subsequent to onset.

Pellagrin 824, aged 17 at onset of pellagra in 1910, suffered a mild recurrence in 1911, apparently during a pregnancy which began in April. Her child was born in December. In 1912, 1913 and 1914 she remained free from recurrence of pellagra.

Pellagrin 750 was 18 years of age when she had her initial attack in 1910. For 1911 and 1912 there are no definite records of recurrence. In July, 1912, she became pregnant and gave birth to a child in March, 1913. The following August she suffered a mild recurrence and again had a renewal of symptoms in 1914.

Pellagrin 275 had her first attack of pellagra in the spring of 1910, when she was 18 years old. She has had a recurrence each year thereafter up to and including 1914. In 1913 she became pregnant in March, suffered a mild recurrence of pellagra in June and was delivered the following December. This is an instance of recurrence of pellagra coincident with pregnancy during the summer season.

Pellagrin 1153, aged 19 at the time of onset of pellagra in the summer of 1910, remained entirely free from symptoms of pellagra during the four years subsequent. In 1913 a childbirth was recorded, but the season of pregnancy was not noted.

Pellagrin 702, aged 23, suffered her first attack in October, 1910. She had recurrences each year thereafter. She gave birth to a child in April, 1913, and suffered a recurrence of pellagra in May, 1913.

Pellagrin 544, aged 23, suffered her first attack in the fall of 1910, about one month after childbirth. In 1911 she was free from symptoms. She also gave birth to a child in the summer of that year, but the exact month of delivery is not recorded. In 1912 she was also free from recurrence of pellagra, but in 1913 after giving birth to a child in April she promptly developed pellagrous symptoms. For 1914 the record is uncertain.

Pellagrin 112, aged 24, first developed symptoms of pellagra in June, 1910, a few weeks after childbirth. In 1911 she suffered a recurrence and also had an induced abortion at six months in December. She remained free from symptoms during 1912, again becoming pregnant in December, 1912. In April, 1913, she suffered a mild recurrence while still pregnant. The child was born in August of the same year. In 1914 the patient remained free from symptoms.

Pellagrin 218, aged 25, had her initial attack in the summer of 1910, the exact month of onset being unrecorded. In June, 1910, she became pregnant, giving birth to a child in February, 1911. Early in 1911 she had a recurrence of pellagra, but the exact date is not recorded. In April of the same year she again became pregnant and the child was born in January, 1912. The following March she suffered her second recurrence. In May, 1913, pellagrous symptoms again developed. In July, 1913, she became pregnant a third time, and five weeks after delivery, on March 28, 1914, she suffered her fourth recurrence of pellagra. Here, then, are three instances of recurrence following within three months after childbirth.

Pellagrin 282, aged 26, had her initial attack in September, 1910. She had a recurrence in September, 1911, but in 1912 she remained free from symptoms.

In 1913, after a childbirth in July, she had a recurrence of pellagra with two eruptions of the erythema, and she also had a renewal of symptoms in 1914.

For history of Pellagrin 95 see section on Initial Attack During Pregnancy.

Pellagrin 127, aged 28 at onset of pellagra in 1910, suffered a recurrence the following year, but remained free from symptoms in 1912. In 1913 she became pregnant in May and escaped recurrence that year. In 1914, after a childbirth in February, she suffered a recurrence of symptoms in the early summer.

Pellagrin 171 was 28 years old when she had her initial attack of pellagra in October, 1910. She had a recurrence in April, 1911, but in 1912 she was pregnant from January and the symptoms of pellagra did not appear. She was again pregnant from January, 1913, and remained free from the disease during that year. In 1914, however, she had a mild recurrence.

Pellagrin 279 was 29 years of age at onset of pellagra in July, 1910. She had a recurrence in 1911, but remained free from symptoms during 1912 and 1913. In February, 1914, she became pregnant and escaped recurrence in that year to the date of the last observation, July 20, 1914.

For history of Pellagrin 18 see section on Initial Attack During Pregnancy.

Pellagrin 860, aged 32, developed her first erythema in August, 1910, shortly after childbirth. During the three following years she remained free from symptoms. In February, 1914, she suffered a severe recurrence about five weeks before the birth of her child and she died of pellagra two months after delivery.

Pellagrin 6, aged 32, had her initial attack in April, 1910. The following October she became pregnant and remained free from symptoms in 1911, being delivered in June. In 1912 she had a recurrence and died Aug. 21, 1912.

Pellagrin 29, aged 40 at the time of onset in 1910, suffered a recurrence in 1911 and 1912. In 1913 she remained free from symptoms and became pregnant in the fall of the same year. She gave birth to a child in May, 1914, and escaped recurrence during that year also.

There are, therefore, among the white pellagrins incident in 1910 nineteen instances of pregnancy subsequent to onset of pellagra. Seven of these terminated early in the year, five in the summer, six in the fall months and for one the exact season was not recorded. Of the pregnancies with termination in the spring, five were quite free from manifestations of pellagra, a severe recurrence in the eighth month was suffered in one and for the seventh there was no definite history. Of the five pregnancies with childbirth in the summer, four were free from symptoms and a mild recurrence in the fifth month of gestation occurred in the fifth case. Of the six instances of pregnancy with fall termination, three were entirely free from symptoms, mild recurrences in the early months were suffered in two and for the sixth there was no definite history.

Among the white pellagrins incident in 1910 there were two cases of onset during pregnancy and one case in which the onset month was uncertain, with subsequent childbirth. For these three cases of childbirth subsequent to onset, as well as for eighteen of the nineteen instances of pregnancy just mentioned above, the pellagrous history of the mother following delivery may be considered. For one 1914 pregnancy subsequent history is not available, because our field observations ceased in the fall of that year. Of the twenty-one instances

of childbirth subsequent to initial attack of pellagra in 1910, therefore, eight occurred in the spring, five in the summer, seven in the fall and for one the exact season was not recorded. In six instances of delivery in the spring a recurrence of pellagra appeared within three months after childbirth, in one case the mother remained free from symptoms and in one instance a severe recurrence during late pregnancy persisted till death two months after delivery. Of the five instances of childbirth in the summer, all but one were free from recurrence of symptoms during the following three months. Of the seven instances of childbirth in the fall and winter, none was followed by recurrence of pellagra within three months.

There are twenty-two white pellagrins with initial attack in 1911 who have a record of pregnancy or of childbirth subsequent to onset of pellagra.

Pellagrin 896 was 19 years old when she had her first attack in April, 1911. In the following September she became pregnant and gave birth to a daughter in May, 1912. Near the termination of this pregnancy, in April, 1912, she suffered a mild recurrence of pellagra. She had recurrence also in 1913 and 1914.

For history of Pellagrin 62 see section on Initial Attack During Pregnancy.

Pellagrin 269, aged 23, suffered her first attack of pellagra in May, 1911, just three weeks after the birth of her child. She had a recurrence in May, 1912. That same year she became pregnant in November and the following spring she suffered a mild recurrence before her child was born. She also had a recurrence in 1914.

For history of Pellagrin 568 see section on Initial Attack During Pregnancy.

For history of Pellagrin 724 see section on Initial Attack During Pregnancy.

Pellagrin 726 was 25 years old when she suffered her first attack of pellagra in May, 1911. In January, 1912, she became pregnant and she remained free from pellagrous symptoms during that year. She also escaped recurrence in 1913, but in June, 1914, while she was again pregnant, she suffered a mild recurrence of symptoms three weeks before the birth of her child.

Pellagrin 678, aged 26 at onset, suffered her initial attack in the summer of 1911, following a childbirth in March. In February, 1912, she again became pregnant, giving birth to a son in October. She remained free from symptoms of pellagra during that entire year and she remained free from recurrence of the disease up to the last observation in May, 1914.

Pellagrin 1033, aged 27 at onset, had her initial attack in the spring of 1911. The year following she suffered a recurrence in March. In September, 1912, she became pregnant and the following spring mild pellagrous symptoms again appeared before the birth of her child in May, 1913. She also had a recurrence in 1914.

Pellagrin 122, aged 27, suffered her initial attack the latter part of March, 1911, in which month she had given birth to a child. In December, 1911, she again became pregnant and escaped recurrence of the disease during 1912. She also remained free from symptoms during 1913 and 1914.

Pellagrin 21 was 27 years of age when she suffered her first attack in May, 1911. The following July she became pregnant and after a childbirth in March, 1912, she had a recurrence of pellagra in May. In 1913 she also had a recurrence in March, which subsided by July. This patient died March 4, 1914, without recurrence.

Pellagrin 879 had her initial attack in April, 1911, when she was 29 years of age. In March, 1912, she gave birth to a daughter. She escaped recurrence

during 1912. She also remained free from symptoms of pellagra in 1913, in which year she again became pregnant in April. She suffered a recurrence of pellagra in April, 1914.

Pellagrin 281 was 32 years of age at the time of onset of pellagra in July, 1911. In February, 1912, she became pregnant and escaped recurrence for that year. She also remained free from symptoms in 1913, but suffered a recurrence in 1914.

Pellagrin 84, aged 32 at the time of her initial attack in 1911, became pregnant in October of that same year. She suffered a recurrence in July, 1912, only a few weeks after the birth of her child. In 1913 and 1914 she was free from symptoms.

Pellagrin 822 was 33 years of age when she suffered her first attack in 1911. She escaped recurrence in 1912, in which year she became pregnant in July. After a childbirth in March, 1913, she also escaped recurrence for that year. In June, 1914, the pellagrous symptoms again appeared.

Pellagrin 274 suffered her first attack in June, 1911, when she was 33 years of age. She became pregnant early in 1912 and remained quite free from symptoms during that year. In 1913 she also escaped recurrence, but in 1914 she had a recurrence of pellagra and she died with complications in September, 1914.

Pellagrin 1137 was 34 years of age when she had her initial attack in the spring of 1911. This patient gives a history of the appearance of pellagrous symptoms after childbirth in 1911 and 1913. The years during which she did not bear children, namely, 1912 and 1914, she remained free from recurrence of the pellagrous symptoms.

Pellagrin 1038, aged 34 at onset of the disease in the summer of 1911, remained free from recurrence during the two years following, although her general health was poor in 1912. In 1913 she gave birth to a child in September, and she was not well thereafter. She suffered a recurrence of pellagra in July, 1914.

Pellagrin 599 had her initial attack in 1911, when 36 years of age. She remained free from symptoms the following year, but suffered a recurrence in July, 1913. In January 1914, she became pregnant and remained free from symptoms during that year up to our last observation in June.

Pellagrin 565, aged 36 at onset in the spring of 1911, suffered a recurrence in April of the following year. In May, 1913, while she was pregnant, she had a mild recurrence of pellagra. The record of this case for 1914 is incomplete.

Pellagrin 604 had her initial attack in the spring of 1911, when 37 years of age. She escaped recurrence in 1912 and also in 1913, in which year she gave birth to a daughter in May. In 1914 she suffered a mild recurrence of pellagra in June.

Pellagrin 212, aged 40 at onset in May, 1911, was pregnant throughout the spring and summer months of 1912. She escaped recurrence during that year and remained entirely free from symptoms in 1913 and 1914.

Pellagrin 278 was 40 years of age when she suffered her initial attack in July, 1911. She had recurrences in 1912 and 1913. In January, 1914, she became pregnant and remained free from symptoms up to our last observation, July 20, 1914.

Of the twenty-two instances of pregnancy subsequent to onset of pellagra in 1911, three terminated in the early part of the year, seven in the summer, eleven in the fall and for one the season was not recorded. In all three of the instances of childbirth in the early part of the year the patient remained free from symptoms of pellagra during the period of pregnancy; in four of the seven instances of termination in the summer mild recurrences were suffered late in pregnancy; of the eleven instances of childbirth in the fall, there were

two with summer recurrence during pregnancy, one mild and one pronounced, while nine were free from symptoms.

Among the white pellagrins incident in 1911 there were three who were pregnant at the time of onset of pellagra. For these three instances of childbirth subsequent to onset, as well as for the twenty-two instances of pregnancy subsequent to onset cited above, the history of the mother in the three months following delivery may be considered. Of these twenty-five instances of childbirth, four occurred in the early months of the year and for only one of these was there history of recurrence of pellagra within three months. Of the nine instances of childbirth in the summer, only three were followed by recurrence within three months. Of the eleven instances of a delivery in the fall months, nine were recorded as free from symptoms in the months immediately following, while for two 1914 cases the records were incomplete because our field observations ceased in the fall of that year.

There were sixteen white pellagrins who suffered their initial attack in 1912 and whose records of childbirth or of pregnancy in subsequent years are sufficiently definite to be utilized in this discussion.

Pellagrin 593 was 16 years old at the time of onset in August, 1912. She was pregnant throughout the spring and summer months of 1913, giving birth to a child in August, 1913. She escaped recurrence in 1913, but suffered a mild attack of pellagra in 1914.

Pellagrin 55 had her initial attack in June, 1912, when 19 years of age. She remained free from symptoms of pellagra in 1913, during which year she was pregnant from April. Her history for 1914 is incomplete.

For history of Pellagrin 280 see section on Initial Attack During Pregnancy.

Pellagrin 596 gave birth to a child in December, 1911. The following spring (1912) when she was 19 years old, her first attack of pellagra developed. She suffered a recurrence of the disease in 1913. In February, 1914, she became pregnant again and remained free from symptoms of pellagra for the year 1914 to our last observation in June.

Pellagrin 884 suffered her initial attack of the disease in the summer of 1912, when she was 20 years of age. The following summer she had a severe recurrence of pellagra. She became pregnant in June, 1913. The childbirth in March, 1914, was followed by a severe recurrence of symptoms in May.

Pellagrin 562, aged 23 at onset, suffered her initial attack of the disease in the spring of 1912, the exact month of onset being unrecorded. In April, 1912, she became pregnant, giving birth to a child the following December. She had a recurrence of pellagrous symptoms in May, 1913. In 1914, however, she was pregnant until well into July and remained free from symptoms up to the time of our last observation in the early part of July, 1914.

Pellagrin 102 was 24 years of age when she had her initial attack in May, 1912. She remained quite free from symptoms in 1913. In March, 1914, she became pregnant and had also escaped recurrence in that year to the date of our last observation, in October, 1914.

Pellagrin 833, aged 26 at onset in April, 1912, became pregnant in November of the same year. In May, 1913, while she was still pregnant, a mild recurrence of pellagra developed. She suffered recurrence also in 1914.

Pellagrin 614, aged 27 at onset, suffered her first attack in September, 1912,

shortly after the birth of a child. In 1913 and 1914 she had complete but mild recurrences. In 1914 the symptoms appeared in March, when she was five months pregnant.

For history of Pellagrin 531 see section on Initial Attack During Pregnancy.

Pellagrin 164, aged 30, gave birth to a child in April, 1912. Pellagrous symptoms developed for the first time in the following June. She became pregnant again in June, 1913, giving birth to a child in February, 1914. She remained free from recurrence during 1913 and 1914.

For history of Pellagrin 701 see section on Initial Attack During Pregnancy.

Pellagrin 439 suffered her initial attack in April, 1912, at the age of 32 years. She had a recurrence of symptoms in April, 1913. Through the spring and early summer of 1914 she was pregnant and escaped recurrence during that year to our last observation, May 25, 1914.

Pellagrin 721, aged 32 at time of onset, suffered her initial attack in 1912, the exact month being unrecorded. She became pregnant in July, 1912, and gave birth to a child in April, 1913. In September of the same year she suffered a recurrence, but remained free from symptoms up to the time of our last observation in May, 1914.

For history of Pellagrin 253 see section on Initial Attack During Pregnancy.

Pellagrin 813, aged 35 at time of onset in 1912, became pregnant in February, 1913, and suffered a mild recurrence the following summer. For 1914 the record of this case is uncertain.

There were, therefore, eleven instances of pregnancy subsequent to onset of pellagra among white women with initial attack in 1912. Of these, two terminated in the early part of the year, in one instance of which there was no recurrence of symptoms, while the history in the other was indefinite. There were five instances of pregnancy with termination in the summer, in three of which the patient remained quite free from symptoms, while in the other two, mild recurrences of pellagra appeared during pregnancy. Of the four instances of termination in the fall, the patient remained free from symptoms in three, while in the fourth instance a mild recurrence appeared during pregnancy in the summer.

Among the white pellagrins incident in 1912 there were four with initial attack during a pregnancy and two with subsequent childbirth, but for whom the onset month was indefinite. These six cases of childbirth following onset may be added to the eleven instances of pregnancy subsequent to onset, giving seventeen instances of childbirth subsequent to onset of pellagra. Of these, five occurred in 1914 too late for subsequent observation in our field work, which terminated in the fall of that year. Of the remaining twelve instances of childbirth, six occurred in the early part of the year, a recurrence following within three months in three, while in the other three cases the mother remained quite free from symptoms. In three instances the childbirth occurred in the summer and in each case the mother escaped recurrence of symptoms in the three months immediately following. The three remaining instances of delivery occurred in the fall, the mother remaining free from recurrence for three months subsequent in each case.

Of the white pellagrins who suffered an initial attack in 1913, there are eleven with definite record of pregnancy or of a childbirth subsequent to onset.

Pellagrin 613 was 21 years old when she suffered her initial attack of pellagra in 1913. She was pregnant during the spring and early summer of 1914, being delivered prematurely in July. In this case the delivery at the seventh month of pregnancy did not seem to lessen her apparent resistance to attack during that year, for she remained quite free from symptoms of pellagra up to our last observation, July 20, 1914.

For history of Pellagrin 1167 see section on Initial Attack During Pregnancy.

Pellagrin 600, aged 26 at onset in July, 1913, became pregnant late in the fall. In 1914 she remained free from recurrence during the period of pregnancy. After giving birth to twins in July, she suffered a mild recurrence of pellagra in August.

Pellagrin 873 was 27 years of age when she had her initial attack in 1913, the exact month of onset being unrecorded. In the spring of the same year she became pregnant, giving birth to a child in January, 1914. In April, 1914, she suffered a recurrence of symptoms.

Pellagrin 498, aged 28 at onset, gave birth to a child about three months before the pellagrous symptoms first appeared in 1913. In January, 1914, she again became pregnant and remained quite free from recurrence for that year to the date of the last observation, Sept. 12, 1914.

Pellagrin 997 had her first attack in July, 1913, when 29 years of age. She became pregnant in February, 1914, and remained free from symptoms for that year to the date of the last observation, July 20, 1914.

Pellagrin 537, aged 29 at the time of onset in March, 1913, had been in poor health following her last confinement in October, 1912. In January, 1914, she again became pregnant and escaped recurrence of pellagrous symptoms during 1914 to the date of our last observation, Sept. 2, 1914.

Pellagrin 450 suffered her initial attack in the spring of 1913, when 30 years of age. In 1914 she was pregnant and remained quite free from symptoms up to the last observation in June.

Pellagrin 1207, aged 31 at the time of onset in 1913, became pregnant in the latter part of that year and gave birth to a child in June, 1914. She, however, did not escape pellagrous symptoms in 1914, and suffered a mild recurrence in the spring, while still pregnant.

Pellagrin 889 suffered her first attack in May, 1913, when 31 years of age. One week after the birth of a daughter in April, 1914, she suffered a recurrence of pellagra.

Pellagrin 518, aged 39 at the time of onset in February, 1913, remained free from symptoms in 1914. She was pregnant during the greater part of the pellagra season, giving birth to a child in June or July, 1914.

For histories of Pellagrins 1267 and 964, with onset in 1914, see section on Initial Attack During Pregnancy.

Among the white pellagrins with initial attack in 1913 there were, therefore, nine instances of subsequent pregnancy, of which one terminated in the spring, five in the summer and three in the fall. In the case of the pregnancy with termination in the spring the patient remained entirely free from symptoms of pellagra during the period of gestation. Of the five pregnancies with childbirth in the summer, there was entire freedom from symptoms in four, while in the fifth a mild recurrence of pellagra appeared in the seventh month. For the

three pregnancies with termination in the fall there was apparently complete freedom from symptoms of pellagra during pregnancy.

There was one instance of onset during pregnancy in 1914 and one of uncertain onset date with subsequent childbirth. Adding these two cases of childbirth subsequent to onset to the foregoing nine instances of pregnancy following onset, we have eleven instances of childbirth subsequent to initial attack of pellagra for consideration. Six of these instances of childbirth occurred in 1914, too late for subsequent observations. Of the remaining five, two occurred in the early months of the year, with recurrence of pellagra following in each case; and the other three took place in the summer, a recurrence following in one case, while in the other two the mother escaped recurrence in the three months immediately following.

Among the white pellagrins with initial attack previous to 1915, there were, therefore, eighty-two instances of pregnancy subsequent to onset and eighty-five instances of childbirth after onset of pellagra.

Of the colored pellagrins who suffered the initial attack previous to 1915, there are only four with definite history of pregnancy or childbirth subsequent to onset.

Pellagrin 109 had her first attack in June, 1911, when she was 29 years old. A childbirth in April, 1912, was followed by puerperal fever, and in May the pellagrous symptoms again appeared. A second childbirth in the summer of 1913 was likewise followed by a recurrence of pellagra, of which the patient died in the fall of 1913.

Pellagrin 67, aged 15 at the time of onset in May, 1912, gave birth to an illegitimate child in May, 1913, and suffered a severe recurrence of pellagra, which appeared before accouchement and persisted for several weeks afterward. In 1914 she had another severe recurrence of pellagra of which she died in June, 1914.

Pellagrin 183 was 17 years old at the time of onset of pellagra in June, 1912. She was pregnant during the spring and summer of 1913 and gave birth to a child on Sept. 18, 1913. During 1913 she remained free from recurrence of the disease. She is without record for 1914.

Pellagrin 733, aged 27 at onset of pellagra in 1913, gave birth to a child on May 20, 1914, and appears, from her record, to have remained free from recurrence during that year.

There are in this small group five instances of pregnancy subsequent to onset among colored pellagrins, one terminating in the spring, three in the summer and one in the fall. In four instances the patient remained quite free from symptoms during the period of gestation; in the fifth case a severe recurrence was suffered late in pregnancy and the symptoms persisted after the birth of the child in May. For four of these five instances of pregnancy there is history during the months subsequent to delivery. In one 1914 case further data are not available. Recurrence within three months appeared after the two childbirths which occurred in the spring and summer, respectively. In the case of one summer childbirth, symptoms which had appeared

during pregnancy persisted for several weeks afterward. For the instance of delivery in the fall there was no record of recurrence within three subsequent months.

In the records of all these pellagrins there were eighty-seven instances of pregnancy beginning subsequent to onset of pellagra and there were eighty-eight instances of childbirth taking place subsequent to the initial attack of the disease. The instances in these two categories are not equal in number, because the first contains sixteen[7] instances of pregnancy during 1914 for which there are no records for the months following childbirth, and which, therefore, cannot be utilized in the second category. Moreover, the second group includes not only fifteen of the twenty cases of onset during pregnancy for which there is history subsequent to delivery, but also four cases in which it is uncertain whether the initial attack occurred before or during pregnancy, but for which subsequent history of childbirth and puerperium is available. Furthermore, two instances of pregnancy with recurrence can be utilized in the first category, but must be omitted from the second, since the recurrent pellagrous symptoms persisted for many weeks after delivery in each case.

RELATION OF PREGNANCY TO RECURRENT PELLAGRA

Those patients who survived the initial attack of pellagra present definite records of eighty-seven instances of pregnancy subsequent to the onset of pellagra. Eighty-two of these pregnancies occurred in white women. In three of these the record in regard to recurrence of pellagra is uncertain. In seventeen of the remaining seventy-nine instances, or 21.5 per cent., a recurrent attack of pellagra appeared during the pregnancy, while in sixty-two instances, or 78.5 per cent., the disease did not recur during pregnancy. The colored race presents five instances of pregnancy, a recurrence of pellagra appearing during pregnancy in only one instance, 20.0 per cent. of the five. Table 6 shows a summary of these observations. It is at once evident that the pregnant pellagrins have been very much less liable to recurrence than pellagrous women in general, for whom, as was shown in the preceding paper of this series, the annual recurrence rate was 63 per cent.

The data in regard to the eighteen recurrences of pellagra during pregnancy are summarized in Table 7. In these eighteen recurrences there was not one instance of death from pellagra during the period of pregnancy. In one case, however, the mother died of pellagra shortly after the birth of her child. In three other cases the attack was

7. For ten of these instances of pregnancy in 1914 no recurrence was recorded to the date of the last observation late in the respective period. We feel justified, therefore, in utilizing these in the pregnancy group.

TABLE 6.—Behavior During Pregnancy of Pellagrins Who Had Previously Suffered the Initial Attack

Race	Record Uncertain	Record Definite	Recurrent Attacks		Escapes from Recurrence		Total
			Number	Per Cent.	Number	Per Cent.	
White	8	79*	17	21.5	62	78.5	82*
Colored	0	5	1	20.0	4	80.0	5
Total	8	84*	18	21.4	66	78.6	87*

* This number includes ten pregnancies in 1914 in which no recurrences were noted to the date of the last observation.

TABLE 7.—Summary of the Eighteen Recurrent Attacks of Pellagra Which Occurred During Pregnancy

Pellagrin Number	Age at Recurrence	Date of Recurrence	Month of Pregnancy	Character of Attack	History During Pregnancy
987	26	Spring, 1909	Late	Mild	Recovery
987	29	Spring, 1912	Early	Mild	Recovery
80	28	July, 1911	Eighth	Mild	Recovery*
824	18	1911	Early	Mild	Recovery
275	21	June, 1913	Fourth	Mild	Recovery
112	27	April, 1913	Sixth	Mild	Recovery
980	26	February, 1914	Eighth	Severe	Persisted†
896	20	April, 1912	Eighth	Mild	Recovery
62	23	Summer, 1913	Middle	Severe	Recovery
269	25	May, 1913	Seventh	Slight	Recovery
726	28	June, 1914	Ninth	Mild	Recovery
1023	29	April, 1913	Eighth	Mild	Recovery
565	28	May, 1913	Seventh	Mild	Recovery
833	27	May, 1913	Seventh	Mild	Recovery
614	29	March, 1914	Fifth	Mild	Recovery
813	26	Summer, 1913	Late	Slight	Recovery
1207	22	April, 1914	Seventh	Mild	Recovery
67	16	Spring, 1913	Late	Severe	Persisted‡

* Some weeks after childbirth in August, the erythema reappeared on the hands.

† The attack of pellagra persisted after the birth of her child and the patient died of pellagra in June, 1914.

‡ The attack of pellagra appeared before childbirth and persisted for a considerable time afterward, with recovery. The patient died in a subsequent recurrence of pellagra in June, 1914.

undoubtedly severe, although followed by complete recovery for that year. One of these three patients, Pellagrin 80, gave a history of only the one recurrence after her initial attack in 1909. In the case of another, Pellagrin 62, the disease recurred regularly each year after onset in 1911; while in the case of the third, Pellagrin 67, a colored woman, the second recurrence in the year following the childbirth ended in death. There remain fourteen instances, or 77.8 per cent. of the total number of recurrences during pregnancy, in which the attack of pellagra was mild.

TABLE 8.—DISTRIBUTION OF RECURRENCES OF PELLAGRA DURING PREGNANCY ACCORDING TO MONTH OF GESTATION

	Definite Month									Month Uncertain		
Period of gestation.....	1	2	3	4	5	6	7	8	9	Early	Middle	Late
Number of recurrences.....	0	0	0	1	1	1	4	4	1	2	1	3

The distribution of the recurrent attacks according to the month of gestation is shown in Table 8. The bulk of the attacks of pellagra appeared after the sixth month. Furthermore, the three severe attacks observed are recorded as occurring in the middle of the period of

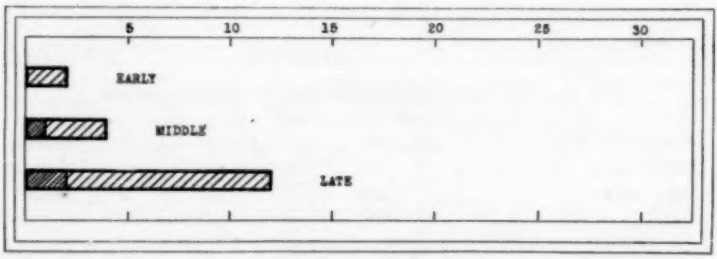

Fig. 3.—Recurrent attacks of pellagra during the first, second and last three months of pregnancy. The total length of the bar indicates the number of instances of recurrence beginning in the respective three-month period. The more thickly shaded portion indicates the number of instances in which the attack of pellagra was severe.

gestation, late in pregnancy and in the eighth month of pregnancy, respectively. It would appear, therefore, that the protective influence of pregnancy is greater in the earlier months.

In Table 9 the eighty-seven instances of pregnancy in pellagrins are summarized according to the season of the year in which the pregnancy terminated or was expected to terminate. From this tabulation it is evident that the season of the year plays a part in determining whether recurrence of pellagra shall take place during pregnancy.

TABLE 9.—Behavior of Pellagrous Women in Respect to Recurrence of Pellagra During Pregnancy of Pellagrins Whose Pregnancies Termi. ated in Different Seasons

Behavior during Pregnancy	White Women		Colored Women		Both Races	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
With pregnancy terminating from January to April, inclusive						
Recurrence	1	6.3	0	0.0	1	5.9
No recurrence	15	93.7	1	100.0	16	94.1
Total definite	16	100.0	1	100.0	17	100.0
Uncertain	2	.....	0	.....	2	.....
Total	18	.....	1	.....	19	.....
With pregnancy terminating from May to August, inclusive						
Recurrence	11	37.9	1	33.3	12	37.5
No recurrence	18	62.1	2	66.7	20	62.5
Total definite	29	100.0	3	100.0	32	100.0
Uncertain	0	.....	0	.....	0	.....
Total	29	.....	3	.....	32	.....
With pregnancy terminating from September to December, inclusive						
Recurrence	5	16.1	0	0.0	5	15.6
No recurrence	26	83.9	1	100.0	27	84.4
Total definite	31	100.0	1	100.0	32	100.0
Uncertain	1	.....	0	.....	1	.....
Total	32	.....	1	.....	33	.....
With time of termination of pregnancy uncertain						
Recurrence	0	0.0	0	.....	0	0.0
No recurrence	3	100.0	0	.....	3	100.0
Total definite	3	100.0	0	.....	3	100.0
Uncertain	0	.....	0	.....	0	.....
Total	3	.....	0	.....	3	.....

When the pregnancy began in the early summer, namely, from April to July, inclusive, and terminated in the period from January to April, inclusive, recurrence of pellagra rarely appeared during the course of the pregnancy. This period, of course, corresponds with the nine months of the year in which onset of pellagra is least frequent in the general population. The pregnancies which began in the period August to November, inclusive, or, roughly, the autumn months, and terminated in the period from May to August, inclusive, showed the greatest frequency of recurrent attacks of pellagra, namely, 37.5 per cent. In these cases the later months of pregnancy coincided with those months of the year in which the attack of pellagra is most usually initiated in the general population, namely, April, May and June. The pregnancies of the third group began in the period from December to March, inclusive, or, roughly, the winter months, and terminated in

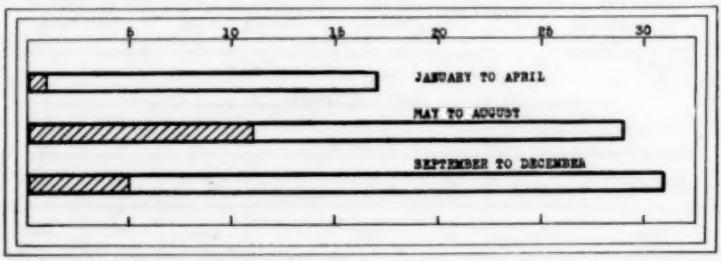

Fig. 4.—Recurrence of pellagra during pregnancy ending in different seasons. The number of instances of childbirth in each four-month period is indicated by the total length of the bar. The number of instances in each group in which recurrence of pellagra appeared during the period of gestation is indicated by the shaded portion of the respective bar.

the period September to December, inclusive. All of these pregnancies extended throughout the pellagra season. Nevertheless, in only 15.6 per cent. of them did recurrence of the disease appear during pregnancy. The months of greatest pellagra incidence, namely, April, May and June, here coincided with the earlier months of pregnancy rather than the later months, so that the low recurrence rate here supports the suggestion that the protective influence of pregnancy is more pronounced in the earlier rather than the later part of the period of gestation.

RELATION OF CHILDBIRTH TO RECURRENT PELLAGRA

There are eighty-eight instances of childbirth among pellagrins for whom there are records subsequent to the childbirth. The behavior during the three months following childbirth in these eighty-eight instances is summarized in Table 10. The frequency of recurrence in

TABLE 10.—Behavior of Pellagrins During the Three Months Following Childbirth

Race	Recurrent Attacks		Escapes from Recurrences		Total
	Number	Per Cent.	Number	Per Cent.	
White	21	24.7	64	75.3	85
Colored	2	66.7	1	33.3	3
Total	23	26.1	65	73.9	88

TABLE 11.—Behavior of Pellagrous Women in Respect to Recurrence of Pellagra During the Three Months Following Childbirth in Different Seasons

Behavior after Childbirth	White Women		Colored Women		Both Races	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
With childbirth occurring from January to April, inclusive						
Recurrence	13	50.0	1	100.0	14	51.9
No recurrence	13	50.0	0	0.0	13	48.1
Total	26	100.0	1	100.0	27	100.0
With childbirth occurring from May to August, inclusive						
Recurrence	7	25.0	1	100.0	8	27.6
No recurrence	21	75.0	0	0.0	21	72.4
Total	28	100.0	1	100.0	29	100.0
With childbirth occurring from September to December, inclusive						
Recurrence	0	0.0	0	0.0	0	0.0
No recurrence	28	100.0	1	100.0	29	100.0
Total	28	100.0	1	100.0	29	100.0
With season of childbirth uncertain						
Recurrence	1	33.3	0	.....	1	33.3
No recurrence	2	66.7	0	.....	2	66.7
Total	3	100.0	0	.....	3	100.0

the first three months after childbirth, namely 26.1 per cent., is enormously high for a period of three months, just one quarter of a year. It may be compared with the annual recurrence rate of 63 per cent. for the total female pellagrins of Spartanburg County after dividing this latter figure by four, which yields a recurrence rate of 15.8 per cent. for a period of three months. It may also be compared with the recurrence rate during the nine months of pregnancy shown in the preceding section of this paper, namely, 21.4 per cent., after this latter figure has been divided by three, giving 7.1 per cent. as the recurrence rate for a period of three months. In comparison with either of these, the recurrence rate in the three months following childbirth, namely, 26.1 per cent., stands out conspicuously.

The behavior of these pellagrous women during the three months following childbirth is shown in Table 11, according to the season of the year in which the childbirth occurred. It is at once evident that

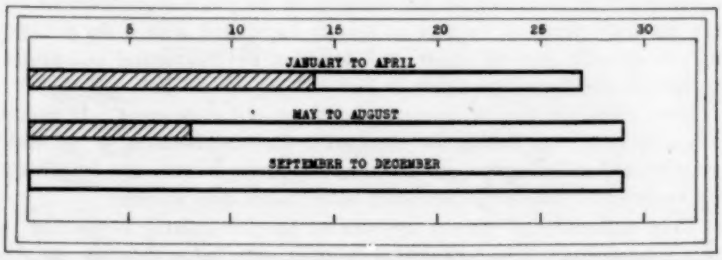

Fig. 5.—Recurrence of pellagra within three months following childbirth in different seasons. The number of instances of childbirth in each four-month period is indicated by the total length of the bar. The number of instances in which recurrence of pellagra appeared in the respective group within three months after childbirth is indicated by the shaded portion. Note that childbirth from September to December was not followed by recurrence of pellagra within three months in any instance.

the pellagrous women who bore children in the period from January to April, inclusive, most frequently suffered a recurrence of pellagra within three months. About one half of such childbirths were followed by recurrence of pellagra within this time. The childbirths in the period from May to August, inclusive, were followed by a recurrence of pellagra within three months in less than one third of the instances. On the other hand, the twenty-nine births which occurred in the period from September to December were not followed by recurrence of pellagra within three months in a single instance. It would appear, therefore, that childbirth does predispose to a recurrence of pellagra to a remarkable degree, but that this predisposing influence is not sufficient to determine recurrence of pellagra in the winter season.

THE RELATION OF CHILDBEARING TO RECURRENCE OF PELLAGRA

In the foregoing sections it has been shown that in the population here studied, pregnancy has exercised an inhibitory influence on the recurrence of pellagra, while childbirth has predisposed to it. Inasmuch as gestation is normally accompanied by childbirth it would be of interest to know their combined influence in respect to recurrence of pellagra. If we add together the percentage of recurrences during the nine months of pregnancy, namely, 21.4, and the percentage of recurrences during the three months following childbirth, namely, 26.1, we obtain a figure, 47.5 per cent., which is an appropriate measure of the recurrence rate for twelve months for puerperal women. This is somewhat lower than the annual recurrence rate for total female pellagrins in Spartanburg County, which was 63 per cent. The figures would indicate that puerperal women are less liable to recurrence of pellagra than other women. It is impossible to decide whether this apparent difference may be due directly to the physiologic exercise of the maternal function or may be merely correlated with it on account of the greater general vigor and superior environmental conditions which the childbearing woman is likely to enjoy. In any case the difference from the general recurrence rate is not very great. It would seem, however, to justify the conclusion that childbearing is not contraindicated in pellagrins, as one might suppose if only the frequency of recurrence following childbirth were taken into account.

The influence of season appears to be particularly important in this connection. The pellagrous women who bore children in the period from January to April, inclusive, suffered recurrence of the disease during pregnancy in 5.9 per cent. of instances and the analogous group suffered recurrence in the first three months after delivery in 51.9 per cent. of instances. The approximate frequency of recurrence for the twelve months beginning with pregnancy was, therefore, the sum of these figures, or 57.8 per cent., for this seasonal group. In the group with childbirth in the period from May to August, inclusive, the recurrence rate during pregnancy was 37.5 per cent. and the recurrence rate in the three months after delivery 27.6 per cent. The sum of these, or 65.1 per cent., represents approximately the recurrence rate for the year beginning with pregnancy, for this seasonal group. For the third seasonal group, in which childbirth occurred from September to December, inclusive, the recurrence rate during pregnancy was 15.6 per cent. and in the three months subsequent to delivery it was 0.0 per cent. The recurrence rate for the twelve months in this seasonal group is, therefore, approximately 15.6 per cent.

This indicated contrast is so marked that it seems worth while to present a separate tabulation, including as definite examples only those

TABLE 12.—Behavior of Pellagrous Women in Respect to Recurrence of Pellagra During the Twelve Months Beginning with Conception, Summarized According to Season in Which Childbirth Occurred

Behavior after Childbirth	White Women		Colored Women		Both Races	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
With childbirth occurring from January to April, inclusive						
Recurrence	10	58.8	1	100.0	11	61.1
No recurrence	7	41.2	0	0.0	7	38.9
Total definite	17	100.0	1	100.0	18	100.0
Uncertain	1	.....	0	.....	1	.....
Total	18	.....	1	.....	19	.....
With childbirth occurring from May to August, inclusive						
Recurrence	16	64.0	2	100.0	18	66.7
No recurrence	9	36.0	0	0.0	9	33.3
Total definite	25	100.0	2	100.0	27	100.0
Uncertain	0	.....	0	.....	0	.....
Total	25	.....	2	.....	27	.....
With childbirth occurring from September to December, inclusive						
Recurrence	5	22.7	0	0.0	5	21.7
No recurrence	17	77.3	1	100.0	18	78.3
Total definite	22	100.0	1	100.0	23	100.0
Uncertain	1	.....	0	.....	1	.....
Total	23	.....	1	.....	24	.....
With season of childbirth not definitely recorded						
Recurrence	1	33.3	0	.....	1	33.3
No recurrence	2	66.7	0	.....	2	66.7
Total definite	3	100.0	0	.....	3	100.0
Uncertain	0	.....	0	.....	0	.....
Total	3	.....	0	.....	3	.....

instances in which the initial attack occurred previous to the pregnancy and in which there is available definite record for both the period of gestation and the three months following delivery. The figures are summarized in Table 12. The differences between the three groups are here similar. Those women who bore children from September to December, inclusive, were only about one third as liable to recurrence during the twelve months including the whole period of pregnancy and the three months following childbirth as were the women who gave birth to children in other seasons of the year.

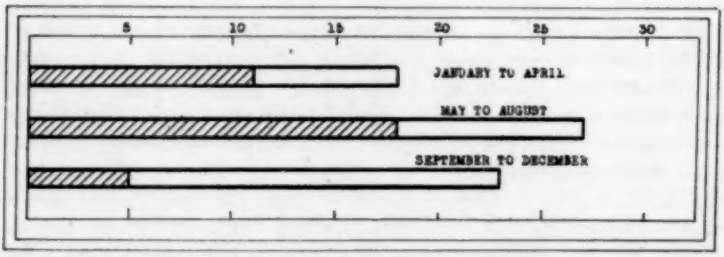

Fig. 6.—Recurrence of pellagra in childbearing women. The number of instances of childbirth in each four-month period is indicated by the total length of the bar. The number of instances in which recurrence of pellagra appeared during the twelve months, including the period of gestation and the three months following delivery, is indicated by the shaded portion. Note that only five of the twenty-three pellagrous women who gave birth to children from September to December suffered recurrence of pellagra during the twelve months.

This third group stands out in remarkable contrast to the other two groups in this respect, clearly indicating that the most favorable season for childbirth in this pellagrous population has been from September to December, inclusive. The contrast is so great that it might well be taken into account by those physicians who have pellagrous married women in their care.

SUMMARY

1. Twenty of the 624 initial attacks of pellagra in women in the age period 12 to 49 years, in Spartanburg County, occurred during pregnancy. This number represents 3.8 per cent. of 624 initial attacks in women of childbearing age and indicates that onset of pellagra has been relatively less frequent during pregnancy than at other times.

2. Sixteen of the 624 initial attacks, or 2.6 per cent., occurred in the month following childbirth, twenty-two, or 3.5 per cent. in the second and third months following childbirth and nineteen, or 3.0 per cent., in the fourth, fifth and sixth months following childbirth.

3. The proportion of initial attacks of pellagra in the six months

subsequent to childbirth is distinctly excessive and the indicated increased liability to the development of pellagra is greater in the earlier months following this event.

4. Among eighty-seven instances of pregnancy in pellagrous women, in only eighteen, or 20.7 per cent., did a recurrent attack of pellagra appear during the period of gestation. This frequency is distinctly lower than the recurrence rate for pellagrous females in general, namely, 63 per cent. per year, and indicates a definitely increased resistance to recurrence of pellagra during pregnancy.

5. Recurrences of pellagra during pregnancy were less frequent in the earlier months. They were usually mild in character and no deaths occurred during the period of pregnancy.

6. In those pregnancies terminating from May to August, inclusive, recurrence of pellagra was relatively most frequent. In these instances the later months of gestation coincided with the season of greatest activity of pellagra.

7. Among eighty-eight instances of childbirth in pellagrous women there were twenty-three, or 26.1 per cent., in which a recurrence of pellagra appeared within three months after this event. This recurrence rate is enormously high in comparison with the normal rate of 15.8 per cent. for three months.

8. The influence of season was evidently of great importance. Childbirth in the period from September to December, inclusive, was not followed by recurrence of pellagra within three months in a single instance. Childbirth in other seasons, on the other hand, was followed by recurrence in about one third of the instances.

9. Considering the period of gestation and the three months following it together as a whole year, we see that recurrence has not been more frequent in childbearing women than for pellagrous women in general.

10. When considered in this way, the instances of pregnancy terminating in childbirth from September to December, inclusive, showed a very low frequency of recurrence for the whole year, namely, 21.7 per cent., which is about one third of the recurrence rate for pellagrous females in general.

11. On the other hand, in those pregnancies terminating during other seasons of the year the frequency of recurrence for the year was between 60 and 70 per cent.

12. These relations are believed to be of practical significance, not only for prognosis, but also as criteria to be utilized in the treatment and management of pellagrous married women.

FURTHER STUDIES ON TYPHOIDIN *

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This paper records the results of further studies on typhoidin made since the publication of the original article of Gay and Force.

Since Gay and Force[1] have reviewed the work on the application of preparations of the typhoid organism to the skin which had been published previous to their article, we shall confine ourselves to a review of observations on typhoidin which have appeared since that date.

The original typhoidin employed by Gay and Force consisted of a ten-day culture of a single strain of *Bacillus typhosus* on glycerin broth evaporated to one-tenth volume. On account of deterioration of the preparation in this form, Gay and Claypole[2] precipitated the original typhoidin with twenty volumes of alcohol, filtered, washed with absolute alcohol and ether, and then dried on porcelain plates over sulphuric acid in a vacuum. With a freshly prepared suspension of this typhoidin powder in phenolated saline, equivalent in concentration to the original typhoidin, these authors were able to produce marked intradermal reactions in previously immunized rabbits, but not in controls. The reaction was characterized by the appearance within twenty-four hours of an indurated, reddened papule, which persisted at the site of inoculation for several days. Gundrum[3] was unable to produce positive reactions in six typhoid cases by the intradermal injection of 0.1 c.c. doses of a commercial typhoid vaccine representing a suspension of 100 million organisms to 1 c.c. Positive results were secured in ten typhoid cases out of thirteen by a similar injection of Gay-Claypole vaccine, representing a suspension of 0.1 mg. (800 million) dried and ground sensitized typhoid organisms to 1 c.c. A red maculopapule appearing at the site between six and forty-eight hours after inoculation was considered positive if it equaled in size the original wheal produced by the intradermal injection.

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1. Gay, F. P., and Force, J. N.: A Skin Reaction Indicative of Immunity Against Typhoid Fever, THE ARCHIVES INT. MED., 1914, **13,** 471.

2. Gay, F. P., and Claypole, Edith J.: An Experimental Study of Prophylactic Immunization Against Typhoid Fever, THE ARCHIVES INT. MED., 1914, **14,** 671.

3. Gundrum: Skin Test in Typhoid, California State Jour. Med., 1915, **13,** 43.

These results are open to the objection that Gay and Claypole[3] have shown that marked reactions may be produced in normal persons by the intradermal injection of a suspension of sensitized vaccine representing from 0.5 mg. to 5 mg. to 1 c.c. In justice to Gundrum, however, it should be stated that he failed to produce reactions in eight controls, and eight of nine malaria patients. Pulay[4] was unable to produce satisfactory results with a typhoidin made from a laboratory strain over a year old. With a typhoidin prepared from a recently isolated strain he secured negative reactions in twenty-eight normal subjects. Positive reactions were obtained in six typhoid patients, thirty-five convalescents, five persons with a history of typhoid long ago, and thirty-nine persons previously vaccinated. In reading the results Pulay lays particular stress on the persistence of the maculopapule, which may be distinct in normals at the end of twenty-four hours, but never after forty-eight hours. In the immunized person, however, the papule may be distinct even on the third day. The preparation used by Pulay was original typhoidin concentrated by evaporation at 40 C. in a vacuum.

Mehler,[5] using original typhoidin, secured negative results in ten normal persons. Positive reactions were produced in eight of ten persons with a history of typhoid (one fifty years, one thirty years, one nineteen years, one fifteen years and again three and one-half years, and four three and one-half years previously) and negative reactions in two persons with a history of typhoid three and one-half years previous to the test. Of ten persons receiving two injections of typhoid vaccine three and one-half years previous to the test, only one gave a positive reaction, while thirteen of twenty persons receiving three injections three and one-half years previous to the test gave positive reactions.

Nichols[6] by cutaneous application of a typhoidin powder reached the following conclusions:

1. The test was negative in 25 per cent. of cases with a history of typhoid, while typhoid fever is supposed to give 95 per cent. immunity.

2. The test was negative in 36 per cent. of those vaccinated under four years previous to the test, while experience has shown that vaccination protects in more than 64 per cent. of cases.

3. A preparation of *B. paratyphosus A* gave 66 per cent, of positive reactions in cases giving 75 per cent. reactions to typhoidin,

4. Pulay: Diagnostiche Hautreaktion bei Typhusrekonvaleszenten, Typhuskranken und Schutzegeimpften mit Typhin nach Gay and Force, Wein. klin. Wchnschr., 1915, **28,** 1189.

5. Mehler: Oration on Medicine—Prophylaxis of Typhoid Fever, Jour. Iowa State Med. Soc., 1916, **6,** 99.

6. Nichols: Antityphoid Vaccination, Jour. Exper. Med., 1915, **22,** 780.

whereas there is no immunity to paratyphoid after typhoid immunization.

4. A positive typhoidin reaction is indicative of a history of typhoid fever or typhoid immunization, but a negative reaction is not necessarily indicative of the opposite condition.

Nichols interpreted his reactions according to the original criteria of Gay and Force; a difference in diameter between typhoidin and control spots of at least 2.5 mm. twenty-four hours after the application of the test. Had he waited for the toxic action to subside (forty-eight hours) his results with paratyphoidin might have been different. His results with typhoidin are practically identical with ours, since we obtained negative reactions in 23 per cent. of patients with history of typhoid and 36 per cent. of persons vaccinated under four years previous to the test. We are not prepared, however, to agree with his statement of the percentage of recovered typhoid patients who are protected. Sawyer[7] found a history of previous typhoid in 15 per cent. of cases in an epidemic occurring in Hanford, Calif., while Kelly[8] secured a similar history from 8.2 per cent. of cases at Taft, Calif.

Austrian and Bloomfield[9] employed suspensions of dried typhoidin powders derived from both a single and several strains of *B. typhosus*. These suspensions were injected intradermally in doses ranging from 0.00001 gm. to 0.02 gm. of the dried powder. The original criteria of Gay and Force were employed in interpreting the reactions, which were positive in all of sixty-six cases comprising normal persons, those previously vaccinated, and persons with a history of typhoid. In several cases they report local and general reactions comparable to those following typhoid vaccination. These results of Austrian and Bloomfield were in no way remarkable when the amount of typhoidin used is considered, together with the failure to wait for the subsidence of the irritant effect of the protein. Gay and Claypole have shown that the evaporation of 10 c.c. of typhoidin broth will yield 0.78 gm. of powder, while the evaporation of an equal amount of control broth will yield 0.5 gm. of powder. Not allowing for the increase in typhoid organisms at the expense of the broth, we may assume that any quantity of typhoidin powder contains one-third its weight of typhoid bacilli. The ordinary immunizing dose of typhoid vaccine used by us contains 0.0001 gm. of typhoid bacilli, equivalent to 0.0003 gm. of typhoidin. Doses of typhoidin then ranging from 0.00001 gm. to 0.02 gm. represent vaccinations with from one-thirtieth

7. Sawyer: Ninety-Three Persons Infected by a Typhoid Carrier at a Public Dinner, Jour. Am. Med. Assn., 1914, **63**, 1537.

8. Kelly: Personal communication to the authors.

9. Austrian and Bloomfield: The Typhoidin Reaction, THE ARCHIVES INT. MED., 1916, **17**, 663.

to sixty-six times the ordinary dose of typhoid vaccine. A normal person might be expected to show local and general reactions to the larger dose, while a previously sensitized person might give a similar reaction to the smaller dose. Positive reactions in the previously vaccinated person could easily be confused with local reactions produced by intradermal vaccination of normal persons.

Kilgore[10] has suggested the use of a quotient for recording the results of cutaneous tests obtained by dividing the diameter of the allergic reaction by the diameter of its control. He regards a quotient over 1.5 as positive, basing his opinion on a number of experiments in the application of typhoidin to the scarified skin. He has pointed out the difficulty of securing uniform scarifications and the impossibility of applying to them constant amounts of the powder. Another element of unreliability in the reaction is a strong irritant effect of the powder itself, which tends to overshadow a weaker specific action. In another communication Kilgore[11] has shown that the higher the typhoidin quotient the greater the chance of reaction to vaccination, and that those who react least to vaccination profit most by its administration, if increase in the typhoidin quotient after vaccination be considered evidence of increased immunity. Kilgore also gives experiences with the intradermal method of administering the typhoidin test. The dose used was approximately 0.05 c.c. of a 1 to 100 suspension of dry typhoidin powder, controlled by an equivalent dose of broth powder. Observations were made at the end of twenty-four hours and results recorded in terms of the typhoidin quotient. Kilgore states that there was practically no difference in the average quotients between a group of fifty-three supposedly immune persons and a group of thirty-eight with a negative typhoid history. Some persons in this series complained of malaise and chilliness, together with an arm as sore as if a typhoid vaccination had been taken. As in the case of Austrian and Bloomfield, the results of Kilgore are not surprising when we consider the doses of typhoidin used which he himself admits were probably too large. The ordinary dose of typhoid vaccine being 0.0001 gm. (equivalent to 0.0003 gm. of typhoidin), it is evident why normal persons should be confused with those who are immune and why both groups should complain of local and general reactions on receiving an intradermal dose of unsensitized killed typhoid organisms two-thirds greater than the quantity used in typhoid vaccination. The excessive dose, together with a twenty-four-hour observation, served to effectually mask any variations in sensitiveness which might otherwise have been apparent.

10. Kilgore: The Typhoidin Quotient, THE ARCHIVES INT. MED., 1916, **17,** 25.
11. Kilgore: A Comparison of Two Methods of Vaccinating Against Typhoid Fever, THE ARCHIVES INT. MED., 1917, **19,** 276.

Kolmer and Berge[12] have made a careful comparison of the typhoidin reaction with the agglutinins, bactericidins, and complement fixing antibodies present in the serum of typhoid immune persons. The typhoidin was injected intradermally in doses ranging from 0.0005 mg. to 0.001 mg., and the resulting reaction observed in twenty-four hours. A reaction was regarded as positive "when the typhoidin site alone showed a reaction or when this site had at least twice the area of erythema and a greater edema than the control," as doubtful when the typhoidin site was one-third greater than the control, and as negative when there was "nothing more than trauma or an area of about equal degree in regard to erythema and edema." From observations on forty-two cases these authors conclude "that agglutinins are present in the blood serum of the majority of persons reacting positively in the skin test, but there is no definite relation between the two as either may be in evidence in the absence of the other." They further mention an irritant action of the typhoidin and suggest the possibility of developing a typhoidin free from toxic substances. They found positive reactions in two of ten normal persons, six of ten persons with a history of typhoid, and twelve of twenty-two previously vaccinated. It is possible that these results might have been different had the reactions been observed at the end of forty-eight hours after the irritant effect of the protein had subsided.

THE PREPARATION OF TYPHOIDIN

Reference has already been made to a typhoidin powder which Gay and Claypole prepared by precipitating the original typhoidin with alcohol, washing with absolute alcohol and ether, and then drying on porcelain plates over sulphuric acid in a vacuum. It was anticipated that this dried typhoidin would retain its potency for a much longer period than the original concentrated glycerin broth preparation. In an attempt to avoid the necessity of weighing a small quantity of this powder whenever a fresh suspension was needed, Dr. Claypole prepared for one of us (Force) a number of small amber vials, each containing such a quantity of the powder that the addition of 1 c.c. of phenolated saline would furnish material for a number of tests. As the powder was believed to be stable, no especial precautions were observed in storing the vials, and we were therefore surprised to discover, on examining them some days later, that the powder had become a moist, treacle-like mass. Thereafter no attempt was made to prepare a saline suspension, but the powder was stored in a large vial in a desiccator, and applied directly to the scarified arm by means of the

12. Kolmer and Berge: The Relation of the Typhoidin Skin Reaction to Immunity in Typhoid, Jour. Immunol, 1916, **1**, 409.

chisel used in making the abrasion. The obvious tendency of the powder to absorb moisture, however, raised the question as to the possible hydrolyzing effect of the residual moisture during the rather slow process of drying over sulphuric acid. Studies were therefore made by one of us (Stevens) with a view to developing a method for the rapid dehydrating of the typhoidin powder to a constant weight, experience with other proteins having shown that a rapidly dehydrated preparation is extremely stable.

The method of preparation finally adopted was a modification of the technic of Gay and Claypole. Ten flasks, each containing 25 c.c. of meat infusion broth (1 per cent. peptone), were inoculated with ten different strains of *B. typhosus*, and incubated for ten days. The ten cultures were then poured into a large glazed porcelain dish and the mixture was evaporated for sixteen hours over an acetone bath to one-tenth the original volume. This concentrated polyvalent culture was then transferred to a 500 c.c. stoppered cylinder, thirty volumes of 95 per cent. alcohol were added, the mixture thoroughly shaken and allowed to stand for twenty-four hours. At the end of this period the supernatant fluid was drawn off by means of a pipet attached to a filter pump, the precipitate washed in 95 per cent. alcohol, and allowed to stand until entirely settled. The alcohol was again drawn off and the precipitate washed twice with absolute alcohol, and the precipitate allowed completely to settle in each instance before removing the supernatant fluid. After the removal of the alcohol the precipitate was shaken up with absolute ether, allowed to settle, again shaken, the suspension poured onto a hard filter paper (C. S. & S. 575), and the precipitate again washed with absolute ether, being careful to keep it covered with a watch glass while draining. The covered funnel containing the precipitate was immediately placed in an oven containing an open dish of sulphuric acid, and kept at a temperature of 40 degrees C. for thirty minutes. At the end of this time the watch glass was removed from the filter and the open filter left in the oven for eighteen hours. The filter paper was then removed from the funnel, opened out, and left in the oven until the precipitate crumbled readily. It was then transferred to an agate mortar, ground by hand, placed in a dark-colored, glass-stoppered vial, weighed, and exposed in a desiccator over sulphuric acid. When the powder has dried to constant weight the stopper may be inserted and sealed with paraffin.

The following comments on this method of preparation may perhaps be in order: (1) The different strains of *B. typhosus* used in making the polyvalent typhoidin were selected to represent the various

antigenic properties described by Hooker.[13] (2) The growth in each of the ten flasks was determined to be a pure culture before the strains were mixed. (3) As soon as the suspension of typhoidin in absolute ether is poured onto the filter it should be covered with the watch glass and kept covered, except when more ether is poured on for washing. A few seconds' exposure to the air at this stage will convert the precipitate into a gumlike mass, whereas the particles of typhoidin adhering to the mortar after the grinding show no tendency to deliquescence even after forty-eight hours' exposure. (4) In the absence of a vacuum chamber with thermostatic control, the ordinary gas oven may be used with hot bricks as radiators. The gas flame should be shut off before placing the filter in the oven. (5) Given the ten days' culture, three days should be sufficient for all of the steps, beginning with the concentration of the culture and ending with the exposure of the dry powder in the desiccator. The powder should be dried to constant weight in four days at the most. A period of eight weeks has been found necessary in some instances to prepare dry typhoidin according to the method of Gay and Claypole.

THE TYPHOIDIN TEST AND ITS INTERPRETATION

We have long recognized that the application of typhoidin to the scarified skin is open to the objections so well described by Kilgore. In entering on this series of studies, therefore, we determined to employ the more accurate method of intradermal injection.

Suspensions of typhoidin and its corresponding bouillon control were made in phenolated saline (0.5 per cent. phenol), so that 0.1 c.c. would contain 1 mg., 0.1 mg., or 0.01 mg. of the dried powder in each instance. Three rabbits which had been immunized against *B. typhosus* one year previously and reimmunized six weeks before the test were shaved on the back. On the following day they were inoculated intradermally with 0.1 c.c. of each suspension. Three normal rabbits served as controls. The results of this experiment are shown in Table 1. It will be seen that the dose of 1 mg. of typhoidin is unduly toxic, while the dose of 0.01 mg. did not produce a reaction in the immune animal distinguishable from the reaction in the normal. The medium and low doses were then given to L. R. T., a student, who had been vaccinated within the year. Reference to Table 1 will show that a dilution too high to give definite results in immune rabbits produced a distinct reaction in a previously vaccinated person.

13. Hooker: A Study of the Antigenic Properties of Different Strains of B. typhosus: Preliminary Communication, Proc. Soc. Exper. Biol. and Med., 1916, **13**, 139.

Von Pirquet,[14] Force and Beckwith,[15] and Kolmer and Moshage[16] have shown that irritation due to trauma is reduced to a minimum by intradermal inoculation of 0.05 c.c. of fluid. We therefore finally adopted a dose of 0.005 mg. of typhoidin suspended in 0.05 c.c. of phenolated saline, representing a dilution of 1 to 10,000.

In interpreting the results of the typhoidin test, we have established the following criteria:

1. A positive reaction is indicated by the presence, forty-eight hours after the application of the test, of a maculopapule, with definite erythema measuring at least 5 mm. in one diameter.

TABLE 1.—TESTS TO DETERMINE THE DOSAGE OF TYPHOIDIN

Subject	Dose, Mg.	Reaction First Day		Reaction Second Day		Result
		Typhoidin	Control	Typhoidin	Control	
Rabbit						
1. Immune.....	1	((28.3)	7.6	(19.5)	10.9	Positive
2. Normal.....	1	20.5	7.1	22.9	0	Negative
3. Immune.....	0.1	((17.5)	5	(9.9)	0	Positive
4. Normal.....	0.1	16.8)	0	12.8	0	Negative
5. Immune.....	0.01	(16	7	5.6	2.8	Negative
6. Normal.....	0.01	16	11.8	6.5	0	Negative
Human						
L. B. T., vac. 1915...	0.1	((65.3 ((11.3)	21	((99.8 ((14)	0	Positive
	0.01	(29.9)	25	((76.1 ((9.4)	0	Positive

2. A doubtful reaction is indicated by the absence of either induration or erythema at the end of forty-eight hours.

3. A negative reaction is indicated by the absence of both induration and erythema at the end of forty-eight hours.

Von Pirquet has shown that intradermal inoculations of vaccine lymph produce, in previously vaccinated persons, marked papules which increase in size to the forty-eighth hour and persist even to the seventy-second hour. A good reaction might be produced by this method when an equal amount applied to the scarified skin failed to

14. Von Pirquet: Ueber die verschiedenen Formen des allergeschen Reaction bei der Devaccination, Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1911, **10,** 1.

15. Force and Beckwith: A Laboratory Method for the Diagnosis of Smallpox, Jour. Am. Med. Assn., 1915, **65,** 588.

16. Kolmer and Moshage: A Note on the Occurrence of Pseudoreactions on the Skin, with Special Reference to the Schick Toxin Test, Jour. Am. Med. Assn., 1915, **65,** 144.

produce results. Haring and Bell[17] in observing the intradermal tuberculin reaction consider positive any induration at the point of inoculation larger than the head of a parlor match, increasing in size to the forty-eighth hour and persisting to the seventy-second hour. Gay and Claypole[2] describe the typhoidin reaction on immune rabbits as a red areola with a nodule measuring from 2 to 5 mm. which persists several days after the disappearance of the areola. Force and Beckwith[18] have shown that smallpox vesicle contents inoculated intradermally into previously vaccinated rabbits will produce a red areola and infiltration appearing within twenty-four hours, but which may not reach its maximum until forty-eight hours. Kolmer and Moshage[16] advise the interpretation of diphtheria toxin intradermal reactions on a forty-eight rather than a twenty-four hour basis, though this reaction is not allergic in the sense of the so-called pseudoreaction to diphtheria protein. Levinson[18] would regard no diphtheria toxin test positive unless there was induration measuring at least 5 mm. by 2 mm. and persisting five days with change of color. Weil,[19] in his intradermal tests to detect the presence of pneumonia antibodies, regards the reaction positive when there is an area of erythema and a papule persisting forty-eight hours or more. Combe,[20] in his intradermal tuberculin tests in children, measures the papule present forty-eight hours after the injection of 0.1 mg. of tuberculin. Zingher[21] describes the pseudoreactions obtained by the intradermal injection of diphtheria toxin as anaphylactic responses of the tissue cells to the protein substances of the autolyzed diphtheria bacilli in the toxic broth. The reaction appears early, within from six to eighteen hours, reaches its height in from thirty-six to forty-eight hours and disappears on the third or fourth day, leaving no pigmentation. It is characterized by a small central area of dusky red infiltration, with a secondary areola which shades off into the surrounding skin.

In all of these descriptions of skin tests there seems to be a disposition to recognize the persistence of a well-marked papule to the forty-eighth hour and erythema as more satisfactory criteria for positive reactions than any comparison of allergin with control. In fact in our later studies we have discontinued the use of the control

17. Haring and Bell: The Intradermal Test for Tuberculosis in Cattle and Hogs, Univ. Calif. Pub. Ag. Bull., No. 243, 1914.

18. Levinson: The Value and Limitations of the Schick Diphtheria Reaction, Illinois Med. Jour., 1915, **28,** 405.

19. Weil: Note on a Skin Reaction in Pneumonia, Jour. Exper. Med., 1916, **23,** 11.

20. Combe: Diagnosis of Tuberculosis in Infants, Le Nourrisson, January, 1916, **4,** 73.

21. Zingher: Methods of Using Diphtheria Toxin in the Schick Test and of Controlling the Reaction, Am. Jour. Dis. Child., 1916, **11,** 269.

entirely. Typhoidin represents something more complicated than typhoid bacilli plus peptone broth. At the end of ten days of growth and digestion the organisms have seriously affected the peptone and beef proteins. It follows, therefore, that a powder produced by the evaporation of peptone broth cannot truly serve as a control to typhoidin. Since many persons give marked reactions to peptone broth, it would seem that any attempt to interpret the reaction in terms of the difference between the typhoidin spot and a broth control will lead to much misinterpretation of results.

Our determination to abandon the use of a broth control is in line with the procedure of Zingher,[22] who, in the routine administration of the diphtheria toxin test, has substituted for the control broth a heated toxin, which is administered intradermally to those persons giving a doubtful reaction to the diphtheria toxin. Heating destroys the irritant action of the toxin and any reaction to this product would represent the uncomplicated allergic action of autolyzed diphtheria organisms, that is, the true pseudoreaction. The heated toxin then becomes, for the purpose of this reaction, an actual control to the unheated toxin. Zingher also has observed that persons giving the pseudoreaction, that is, presumably sensitized to diphtheria protein, were much more susceptible to the local action of antitoxin or immunizing injections of toxin-antitoxin. Weaver and Rappaport[23] have noted that persons giving an intradermal pseudoreaction to diphtheria toxin are apt to give a similar reaction to a control of toxin-antitoxin. These authors charge this result to the effect of horse serum, but it is also readily explained on the basis of allergic effects of autolyzed diphtheria organisms. Since Bessau and Schwenke[24] have been unable to remove the allergic effect of autolyzed diphtheria organisms even by boiling diphtheria toxin, it follows that the question of a true control to typhoidin or other cutaneous allergins is still an open one.

SPECIFICITY OF THE TYPHOIDIN REACTION

We have suggested criteria for the interpretation of the typhoidin reaction independent of so-called controls. There remains still the question of specificity. In other words, is the typhoidin reaction due to a toxic effect of the inoculated protein on the tissues, or, even granting such an effect, is there in addition a specific reaction in persons sensitized to typhoid protein? If there is such a reaction how may it be unmasked from the stronger toxic influence?

22. Zingher: The Pseudoreaction in the Schick Test and Its Control, Jour. Am. Med. Assn., 1916, **66,** 1617.

23. Weaver and Rappaport: Further Observations on the Schick Test for Diphtheria Immunity, Jour. Am. Med. Assn., 1916, **66,** 1448.

24. Bessau and Schwenke: Ueber de lokale Diphtherie-Bouillon-Reaktion beim Menschen, Monatsch. f. Kinderh., 1916, **13,** 397.

Stokes[25] holds that many skin reactions are not specific in the sense that they are the results of antigen-antibody activity, but rather the results of the activity of an anaphylatoxin which will produce focal inflammation as the result of the action of the subject's enzymes on his own tissues. This anaphylatoxin is a product of the action of a protease released through parenteral introduction of antiferment adsorbents. On the other hand, Bronfenbrenner[26] claims that more than mechanical action of antiferment adsorbents is involved in these reactions, since there is an element of specificity, not in the ferment, but in the mechanism of its activation. The combination of a specific serum with its corresponding antigen is followed by a change in the

TABLE 2.—DETAILS OF—

Initials	Class	Date of Test	Preparation			
			MAc	MAl	PAe	PAl
M. C.	Normal	2/23/16	#	...	...	...
H. D.	Normal	2/23/16	#	...	...	...
M. S.	Normal	4/ 3/16	...	#	...	...
H. I. S.	History of Typhoid—1906	3/22/16	...	...	...	...
F. B.	1906	3/15/16	#	...	...	...
R. L. S.	Vaccinated 2/21/14, 3 doses; 3/3/15, 2 doses; typhoid 5/25/15	4/26/16	...	...	...	#
D. G.	Previously Vaccinated—					
	2/ 7/14 (3) X	3/ 1/16	#	...	...	...
	3/15/16 (3) Y	4/12/16	...	...	#	...
J. M.	5/12/14 (3) X	2/25/16	#	...	...	...
	5/ 3/16 (2) X	5/ 1/16	...	...	...	#
C. G. P.	9/24/13 (2) X	3/27/16	#	...	...	...
	4/ 3/16 (3) X	4/24/16	...	...	...	#

From February to May, 1916, inclusive.

degree of dispersion of serum colloids, which is in turn followed by the activation of the nonspecific ferment.

While we must admit that parenteral administration of foreign proteins gives rise to certain nonspecific responses, we hope to show in the case of typhoidin that these general reactions tend to subside by the forty-eighth hour; and further, that these reactions are proportional to the concentration of the suspension of typhoidin powder, so that the specific reaction appears to be unmasked by using small doses of the antigen.

25. Stokes: Studies on Intradermal Sensitizations, II. An Intradermal Reaction to Agar and an Interpretation of Intradermal Reactions, Jour. Infect. Dis., 1916, **18,** 415.

26. Bronfenbrenner: Specific Parenteral Digestion and Its Relation to Phenomena of Immunity and Anaphylaxis, Jour. Lab. and Clin. Med., 1916, **1,** 573.

EXPLANATION OF THE TABLES

The accompanying tables, 2, 3 and 4, show the results of typhoidin tests on a group of faculty and students of the University of California.

A uniform dose of 0.05 c.c. of a suspension in phenolated (0.5 per cent. phenol) saline of 0.005 mg. of typhoidin was injected intradermally in each instance. Any injection failing to produce a white wheal with depressions marking the hair follicles was discounted in the estimation of results, but there was no attempt to produce wheals of constant diameter.

—NINE TYPHOIDIN TESTS

Reaction First Day				Reaction Second Day				Reaction Fifth Day				Result
Typhoidin		Control		Typhoidin		Control		Typhoidin		Control		
26.8	5.8	6.5		4.1		4		5.9		4.5		—
(28.7		4.6		5.2		4.6		2		2.9		—
17.3	3.9	10	5	3.6		3.7		3.1		3.1		—
46.1	((8.3)	7.5		56.5	((12.8)	10		10	((5.1)	3.6		—
(27.3	(12.5)	8.9		.....	.....	...			(10)	0		+
10.3		.....			(6.5	...			5)	...		±
11.5	(6.1)	6.1		5		4.9		5.7		3.8		—
.....	.....	.....		(23	((13	...			((5.5)	...		+
37.2	(7.4	3.5		7.8		2.9		0		0		—
(31.5	(10.6)	.....		24.3	(6.4)	...		.....	.....	0		+
(31.6	(7.3)		5)	5.8		3.5		.....	.....	...		—
18.6	(5	7			((9.5)	0			(5)	0		+

Table 2 gives the details of nine typhoidin tests and is presented in order to show the methods employed in recording the results obtained.

The preparations of typhoidin employed were a monovalent strain precipitated with acetone (MAc), a monovalent strain precipitated with alcohol (MAl), a polyvalent strain precipitated with acetone (PAc), and a polyvalent strain precipitated with alcohol (PAl), which was rapidly dehydrated.

In case the person had been previously vaccinated, the date of the last dose of vaccine is given, together with a figure, in parenthesis, indicating the number of doses of vaccine and a letter indicating the type of vaccine used. The types of vaccine which had been employed in the vaccination of this series were U. S. Army (*A*), commercial (*C*), Gay-Claypole (*X*), and a vaccine prepared by the same technic as the latter but unsensitized (*Y*).

TABLE 3.—Summary of Typhoidin Tests

Class	Positive	Doubtful	Negative	Negative, %
Normals	1*	..	17	94
History of Typhoid:				
1899-1904	5	..	5†	50
1905-1910	9	..	1	10
1911-1916	5‡	1§	..	0
Previously Vaccinated:				
1913	25	1	26	50
1914	14	2	13	45
1915	7	1	3	27
1916§	37	7	12	21

* For a history of the case of A. G. see matter under the head "Comment" in the text.
† Four of these gave questionable typhoid histories.
‡ One of these gave a questionable typhoid history in 1915, but had been vaccinated in 1912.
§ For a history of the case of R. L. S. see the case report under the head "Comment" in the text.

TABLE 4.—Reaction to Vaccination (or Revaccination) of Persons Previously Receiving Typhoidin Tests

Class	Result of Test	Persons Reporting after Each Inoculation			Local Reaction						General Reaction					
					Slight			Severe			Slight			Severe		
		1st	2d	3d	1st	2d	3d	1st	2d	3d	1st	2d	3d	1st	2d	3d
Normal	Untested	236	228	105	88	74	30	40	43	9	87	90	19	9	10	2
	Positive	1	....	....	..	..	..	1	..	..	..	..	..	1		
	Negative	9	8	3	3	3	..	1	1	1	2					
Previously vaccinated	Positive	3	1	....	1	..	..	2	..	..	1	..	..	2	1	
	Doubtful	8	3	1	1	..	..	3	1	1	1	..	..	2	1	
	Negative	48	34	21	15	6	3	9	5	..	15	16	5	1	1	
History of typhoid	Negative	5	2	1	..	..	..	1	..	1	..	..	1	1	1	

The measurements of the reactions were made with calipers in the longest diameter and recorded in millimeters. The numbers at the left of each column of measurements refer to the areolae, the numbers at the right to the papules. A well-marked areola is designated by the sign (placed in front of the measurement. A well-marked papule is indicated by the sign) placed after the measurement. If the erythema of the papule is well marked, the sign (is placed in front of the measurement. A double sign indicated great intensity of the reaction. For example: The record 25.4 ((10.6) would indicate an areola of 25.4 mm. not very well marked, with a central papule of 10.6 mm. well marked and very red. Positive reactions are indicated by the sign +, doubtful by the sign ±, and negative by the sign —.

COMMENT

We are, of course, aware of the unreliability of personal statements regarding the presence or absence of a history of typhoid fever. The experience of Sawyer[7] in the Hanford epidemic previously mentioned, has shown the possibility of typhoid fever occurring after a very short incubation period or with slight or indefinite symptoms. The normal persons in our series (Table 3), therefore, have been chosen, with one exception, after elimination of suspicious infections or possible exposure to typhoid fever. The exception noted, A. G., was a physician who, during three years residence in a hospital, was constantly exposed to the disease, though having no memory of an infection. It is interesting to note that A. G. furnished the only positive reaction in our series of normal persons, and our suspicion that he had become sensitized to typhoid protein was further strengthened by a severe local and general reaction following a subsequent dose of typhoid vaccine.

The selection of a group of persons with a history of typhoid is a much more difficult task. The retention of a person in our series (Table 3) was conditioned on a history of one or more attacks of continued fever which had been called typhoid by the attending physician. Whenever in spite of a diagnosis of typhoid the patient's account of the disease contained an element of doubt, we have regarded the history as questionable. It will be seen that on a time basis these doubtful cases tend to group themselves at the beginning of the series before the use of the agglutination test became general.

Table 3 also shows the results of the test as applied to previously vaccinated persons and further classifies these results on a time basis. It will be seen that the percentage of negative reactions grows smaller as the time elapsing from vaccination to test grows shorter. The fact that 21 per cent. of persons vaccinated during the period from February to May, 1916, inclusive, failed to show positive reactions would indicate that the routine three doses of typhoid vaccine are not always enough to sensitize or immunize a person. On the other hand, ten out of thirteen persons showing negative skin reactions before vaccination (or revaccination) gave positive reactions after such vaccination. Of the three persons failing to give a positive reaction after retest, one had received three doses of vaccine within the two months preceding the test, one six doses in the year preceding, and one six doses in the two years preceding.

The insensitiveness of certain persons to typhoid protein is well illustrated by the following case:

R. L. S., a student, after six doses of typhoid vaccine failed to give a positive skin reaction. One month after her last test (eleven weeks after her

last dose of vaccine) she developed typhoid fever, confirmed by blood culture and agglutination tests. She was treated with intravenous injections of Gay-Claypole vaccine and on recovery failed to give more than a very doubtful skin reaction.

This case further illustrates that sensitiveness bears a direct relation to protection.

Table 4 gives a summary of the local and general reactions to vaccination in persons previously receiving typhoidin tests. The reactions are arranged on the basis of response to the typhoidin test. In general it will be seen that the reactions of normal persons (both untested and negative to the skin test) differ very little from the reactions of previously vaccinated persons showing negative typhoidin tests. The revaccination of persons showing complete or partial sensitization, however, was attended by an increase in the severity of the reactions.

It is our growing conviction that typhoid immunity is a much less stable condition than has been generally supposed.[27] Whether, as suggested by Wassermann and Sommerfeld,[28] the reduced susceptibility produced by vaccination is liable to fluctuate under the influence of undernourishment, secondary infection and poor hygienic conditions; or the routine administration of three doses of typhoid vaccine is not a safe rule for every person, the fact is that many persons remain insensitive to typhoid protein after vaccination or lose their sensitiveness in increasing numbers as time elapses. In the words of Gay,[28] "in the case of human beings that have been vaccinated against typhoid fever we have no assurance that they are really protected and still less the assurance as to how long the protection lasts."

Pending further information on the duration of immunity, we have made a routine practice of advising the students of this university to return for a typhoidin test two years after vaccination. Students now being vaccinated (or revaccinated) are advised to return in three weeks for a typhoidin test. All persons showing a negative

27. Reference to our Table 3 will show that we secured negative typhoidin reactions in 50 per cent. of persons who had been vaccinated against typhoid three years previously; in 45 per cent. vaccinated two years previously; and in 27 per cent., one year previously. Lescohier (Duration of Immunity Following Smallpox Vaccination, Jour. Am. Med. Assn., 1913, **61**, 487) has confirmed observations of Kitasato which show that 47 per cent. (48 per cent. Lescohier) of persons successfully vaccinated against smallpox may be successfully revaccinated after three years; 33 per cent. (33 per cent. Lescohier) of persons after two years, and 14 per cent. (28 per cent. Lescohier) after one year. The diminution of immunity following smallpox vaccination, as indicated by susceptibility to revaccination, shows a striking similarity in point of time to the diminution of cutaneous hypersensitiveness to typhoid protein.

28. Wassermann and Sommerfeld: Experimentelle Untersuchungen über die Wirksamkeit der Typhus und Cholera-schutzimpfung, Med. Klin., 1915.

28. Gay: New Uses of Specific Skin Tests in Certain of the Infectious Diseases, Am. Jour. Med. Sc., 1915, **149**, 157.

reaction are revaccinated, for even if the cutaneous sensitiveness to typhoid protein is of shorter duration than typhoid immunity, revaccination on the basis of a negative typhoidin reaction gives the student a safer margin of protection.

SUMMARY AND CONCLUSIONS

1. A stable preparation of typhoidin may be rapidly prepared by precipitating a concentrated broth culture of *B. typhosus* with 95 per cent. alcohol, and subsequent dehydration with absolute alcohol and absolute ether.

2. Definite reactions persisting forty-eight hours were produced in human beings by the intradermal injection of 0.05 c.c. of a 1 in 10,000 suspension of typhoidin in phenolated saline. In order to produce a similar reaction in rabbits, a dose of 0.1 c.c. of a 1 in 1,000 suspension of typhoidin was necessary.

3. It is imperative that no account be taken of the appearance of the reaction at the end of twenty-four hours. A positive typhoidin reaction is indicated by the presence, forty-eight hours after the test, of a well-defined erythematous papule at least 5 mm. in diameter. Out of 108 positive reactions, the forty-eight hour papule measured 10 mm. or over in twenty-six instances; the average measurement of 108 papules was 8.4 mm.

4. Out of eighteen normal persons, seventeen gave negative reactions; out of twenty-six persons with a history of typhoid, nineteen gave positive reactions, one gave a doubtful, and six persons (four with questionable typhoid histories) gave negative reactions. Out of 152 persons previously vaccinated against typhoid, twelve of fifty-six vaccinated during 1916, three of eleven vaccinated during 1915, thirteen of twenty-nine vaccinated during 1914, and twenty-six of fifty-two vaccinated during 1913 gave negative reactions (Table 3).

5. Ten of thirteen persons showing a negative reaction to the skin test before vaccination (or revaccination) gave positive reactions after vaccination (or revaccination).

6. The revaccination of previously vaccinated persons showing negative skin reactions produced local or general reactions no greater in intensity than the vaccination of normal persons, and much less severe than the reactions produced by the revaccination of persons showing positive skin reactions (Table 4).

7. The routine administration of three doses of typhoid vaccine is in many cases not sufficient to produce sensitization to typhoid protein, and presumptively, therefore, protection against typhoid fever.

8. Even granting that typhoid immunity is of longer duration than cutaneous sensitiveness to typhoid protein, the disappearance of this sensitiveness furnishes an indication for revaccination of the person, and still allows a margin of safety within the as yet indefinite limits of typhoid immunity. There is as yet no evidence that a positive typhoidin test is not indicative of protection against typhoid fever. In two instances at least, in our experience, a negative typhoidin test after vaccination was followed by typhoid infection. We feel justified, then, in proceeding on the original assumption of Gay and Force that this test may be used as a measure of protection against typhoid.

ON THE RELIABILITY OF THE WASSERMANN REACTION

A STUDY OF THE SOURCES OF ERROR AND AN ATTEMPT TO STANDARDIZE THE TECHNIC *

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In the last few years several articles[1] have appeared reporting inconsistencies between the results of different laboratories on the Wassermann reactions of identical specimens of blood and tending to throw doubt on the diagnostic value of the Wassermann reaction. To show that this state of affairs was becoming one of great practical significance one need only refer to the fact that in 1915 a committee was summoned by Dr. Haven Emerson, then deputy commissioner (now commissioner) of health of New York City, to consider the whole question of the reliability of the Wassermann reaction, and to attempt to standardize the technic. One of the particular reasons for the formation of the committee was the use of the Wassermann reaction by the department of health in the medicolegal examination of certain persons, such as food handlers. This committee, composed of serologists in charge of Wassermann reactions in large New York institutions, held many meetings and obtained some interesting results, but unfortunately its work was not completed. The chief difficulty encountered was that although each of the workers on the committee was doing a large number of tests, and although each of them felt that he was doing the Wassermann reaction according to classic and well-recognized principles, hardly any two of the dozen members of the committee agreed precisely in the technic for any single step in the reaction.

It should be stated at once that with the exception of Dr. Coca, who has expressed his opinions elsewhere,[2] the members of the committee were unanimously of the belief that in the vast majority of cases the Wassermann reaction is reliable and (in our climate, with exceptions

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1. Wolbarst: New York Med. Jour., 1913, **97,** 378; Interstate Med. Jour., 1915, **19,** No. 2. Pusey: Jour. Am. Med. Assn., 1913, **61,** 921. Stone: New York Med. Jour., 1914, **99,** 1242. Gottheil: Progressive Medicine, Sept. 1, 1914, p. 161. Coca: Bull. Johns Hopkins Hosp., 1916, **27,** 155. Ohle and MacKinney: Jour. Am. Med. Assn., 1915, **65,** 863.

2. Coca: Jour. Immunol., 1916, **1,** 484.

well known and easily excluded) diagnostic of syphilis; that most of the relatively few instances in which contradictory reports were received from different laboratories were cases of actual syphilis having very weak reactions detectable by certain technical methods, but not by others; and that when all possible precautions are taken false positive reactions (that is definite positives, complete or almost complete fixations) are avoidable.

As a member of the committee, I took on myself the task of searching for the origins of some of the variations in technic. I found that different writers on the subject had varied among themselves as widely as did the individual workers in New York City. In fact in trying to get at the truth about such important questions as the superiority of one or another antigen, these variations in technic of different workers offered great obstacles. It therefore seemed necessary that before any serious comparative work on these questions could be undertaken, certain standards of technic would have to be adopted.

The review of the literature attracted me to the study of the sources of error in the reaction. And in the present article it is proposed to present the results of experiments on this subject, as well as to review critically the principal variations from the original technic described by Wassermann. Only those methods will be considered which do not depart very widely from the original Wassermann reaction. Methods of serodiagnosis involving considerable novelty, such as that of Noguchi, of Bauer, of Stern, and of Hecht and Weinberg, however valuable in themselves, will not be discussed. The committee called together by the New York board of health failed to come to an agreement, not so much because of radical differences of opinion, as because it lacked a definite plan for attacking the problem. It is one of the objects of the present study to offer such a plan for future work.

To many readers some of the considerations detailed below may seem so minute as not to warrant serious consideration. Nevertheless, the fact remains that while different laboratory workers can almost invariably detect the strongly positive Wassermann reactions and agree in the vast majority of the definitely normal cases, their results in detecting weakly positive reactions with regularity sufficient to have diagnostic value disagree widely. These irregularities are not accidents, but are due to variations in minor points of technic which are usually considered of little importance.

One of the most discouraging features about any attempt to standardize the reaction or to compare results (such as the attempt made by the committee referred to) is the dogmatic assurance of many workers that the particular method that they use (though they never have tried any other) gives the best results. It is hoped that the pres-

ent paper will help to a more catholic attitude. As a result of personal acquaintance with a large number of workers the author is convinced that the man is more important than the method, and that some workers obtain good results with methods in themselves inferior, because the worker is intimately informed of the peculiarities and limitations of the method he uses. Not a few of the workers, indeed, follow a technic that can be described only as "rule of thumb" and that does not admit of accurate comparison or description, because it depends entirely on the experience of the worker. In fact some who have published descriptions of their methods introduce into their actual work so many personal features that it would be impossible for another worker to follow their printed directions and obtain identical results. Such methods have no advantage and should be dropped for those which admit of precise description and of successful repetition from the description by any competent worker.

The present paper will follow the plan of discussing each source of error and each point on which different workers vary under a separate heading.

THE TOTAL VOLUME OF THE REACTION

Wassermann and his immediate followers, such as Citron and Meier, used each of the five reagents entering into the reaction (patient's serum, antigen, complement, cell emulsion and amboceptor) in such a dilution that 1 c.c. of each was employed and the total volume at the end of the reaction was 5 c.c. For the sake of economy subsequent workers have used smaller amounts, such as one half, one quarter, or one tenth of all the reagents involved, so that the volumes of their reactions were 2.5 c.c., 1.25 c.c., or 0.5 c.c. In the present paper for purposes of comparison the quantities given by all the writers will be translated into terms of the original 5 c.c. volume.

Whatever particular volume is decided on by the worker must be adhered to uniformly in all tubes throughout titrations and tests; otherwise fallacious results are easily obtained, because the activity of complement depends on its concentration and not on the total amount present. This is a point of some importance which does not always receive the attention it deserves. It is suggested that future writers can contribute greatly to the ease of study of the subject if they will describe all their work in terms of the original Wassermann volume, simply stating that the actual quantities used were one-half, one-fourth or one-tenth of those given.

It will be necessary to exclude from consideration in this present study the work of a few authors who, without any special reason, use such arbitrary quantities of the various reagents that their results cannot be compared with those of any one else. It is unfortunate too that many of those who have written on the subject, particularly those who have written statistical or clinical articles, have failed to describe their technic or have described it so imperfectly that their work has little value.

METHODS OF KEEPING THE HEMOLYTIC SYSTEM CONSTANT

The correct adjustment of the hemolytic system is the crucial factor in the technic of the reaction. The strength of the complement in guinea-pig serum is the most variable factor which enters into the Wassermann reaction. If it were not for this it would be easily possible to give definite rules for the quantities of all the reagents. Before discussing the various methods which have been used for adjusting the amounts of the hemolytic system, it will be worth while to review briefly a few of the fundamental facts of their quantitative relationships.

Morgenroth and Sachs[3] showed that within certain limits the amounts of complement and of hemolytic amboceptor needed to lake a given amount of red cells were in inverse proportions, that is to say, the more complement used, the less amboceptor was needed and vice versa. However, a limit is presently reached in each direction, a smallest amount of amboceptor with amounts smaller than which the addition of further complement does not produce complete laking, and a smallest amount of complement beyond which further increases of amboceptor will not produce complete laking. These smallest hemolytic amounts it was proposed by Bordet to call units of amboceptor and complement, with the idea that in any given serum the volumes which contain these units contain actually equal quantities of the active ingredients.

Unfortunately the terms unit of complement and unit of amboceptor have come to be used in several senses, so that they have practically lost their original significance. Most writers have titrated complement with some arbitrary amount of amboceptor, or amboceptor with some arbitrary amount of complement, and have called the minimal hemolytic dose in each case a unit of the respective substance. Probably the most generally accepted definitions are given by Noguchi;[4] the unit of amboceptor is the smallest amount that will completely lake the standard amount of cells in the standard volume of fluid in one hour at 37 C. (in the water bath) in the presence of an excess of complement; and the unit of complement is the least amount of complement which will give complete laking with one such unit of amboceptor. In the present article the term unit will be retained, but it will be specified in each instance what kind of unit is meant.

Certain other factors, namely, the duration and mode (whether in water bath or air thermostat) of incubation, also greatly affect the results, but for the sake of clearness a consideration of these factors will be deferred. Likewise the various technical factors which are believed to affect the strength of complement (such as the mode of obtaining blood, whether the guinea-pigs are etherized or not, the time which the blood is allowed to stand on the clot, etc.) will not be considered here, as the fundamental fact to be faced is that quite without these factors the strength of the complement of different guinea-pigs varies widely and the problem to be solved is how to make allowance for this variation.

Wassermann used a fixed dose (namely, 0.1 c.c.) of fresh guinea-pig serum as complement, 1 c.c. of 5 per cent. sheep cells, and two units of amboceptor. By two units of amboceptor he meant twice that amount of amboceptor which gave complete laking of the amount of cells stated, in one hour, in the air thermostat at 37 C., with the fixed dose of complement. The dose of complement was chosen because it was believed to be an excess, that is, to represent at

3. Morgenroth and Sachs: Berl. klin. Wchnschr., 1902, No. 35, p. 817.
4. Noguchi: Serum Diagnosis of Syphilis.

least two Bordet units (twice the least amount of complement which would give complete laking with a very large excess of amboceptor). Wassermann made allowance for the natural variation in strength of guinea-pig complement by varying the amount of amboceptor, the two units being redetermined by a fresh amboceptor titration each time the tests were made. This method has been adhered to by Citron and many other subsequent workers.

The question of whether to make allowance for the variability in complement strength by varying the amount of amboceptor or by varying the amount of complement (or even by varying the amount of cells, as is done in the Hecht and Weinberg modification of the Wassermann reaction) is a question which has not received adequate consideration, but which seems to have been determined arbitrarily by different workers. A large number of workers (such as Thomsen, Boas, MacIntosh and Fildes, Sormani, Thomas and Ivy, Walker and Swift) use fixed amounts of amboceptor and vary the amount of complement according to its strength. Others (such as Bruck, Citron, Stern, Sachs [Schlossberger]) use fixed complement and varying amboceptor.

There is something to be said for each procedure (varying amboceptor or varying complement). If the practice of keeping a fixed dose of complement and varying the amount of amboceptor gave equally constant results, it would seem to have one considerable practical advantage, namely, that of saving time in the course of a day's work. For since the complement must be in all the tests from the beginning, whereas the amboceptor goes in only after the preliminary incubation, it is possible to set up tests as soon as the complement is prepared, and then while the preliminary incubation is going on, to determine at leisure the proper dose of amboceptor to be used with the particular complement of that day. On the other hand, when the dose of complement is redetermined for each set of tests, the actual setting up of the test must wait until the complement has been titrated.

But while one can undoubtedly produce the same total hemolytic effect by increasing the amount of amboceptor as by increasing the amount of complement, there is grave doubt as to whether the effect in complement fixation is the same. If one attempts to adjust the total hemolytic power by varying the amboceptor instead of the complement, one does not keep constant the actual amount of complement which enters into fixation in the primary incubation, but only the total hemolytic effect of the second incubation. Since the second incubation is merely a test to see how much complement has been absorbed during the first incubation, one deceives oneself if one hopes by varying the amboceptor to keep the complement in the test proper (that is, the first incubation) at a constant level.

For example, if a given positive serum, with a given antigen, contained exactly sufficient of the reacting bodies to bind all the complement present (a quantity of complement which we will represent by

the letter x), and we were to repeat the test with the same antigen and serum, but with an equal dose of a complement of twice the activity (namely, $2x$), then only half of the second complement would be bound; and although according to the titration one would have diminished the amount of amboceptor so as to get the same hemolytic effect with the double-strength complement, nevertheless the antigen positive serum complex would not have been able to bind any more of the complement and there would be incomplete fixation instead of complete fixation as in the first instance.

I have proved this repeatedly[5] by showing that when one carries out complement fixation with a specimen of complement exceeding the average strength, it is possible to detect far greater dilutions of positive serum by using a double laking dose of the complement (as determined with two units of amboceptor measured by previous titration with average complements) than by using a double laking dose of amboceptor (redetermining the laking dose of course with the fixed dose of complement used in the test). In other words, if one attempts to make allowance for a strong complement by using a diminished amboceptor instead of a diminished complement dose, one invariably misses certain weak positive tests. Kromayer and Trinchese[6] have shown the same thing by what might be called a reductio ad absurdum; they demonstrated that any positive serum will give a negative reaction if a large enough dose of complement be used, but with ordinary doses of complement (after fixation has once occurred) one cannot produce hemolysis (a negative reaction) by the addition of even enormous doses of amboceptor.

It can readily be seen, therefore, that although superficially these two methods of titrating appear to give rather similar results, nevertheless the choice of one or the other indifferently by various workers has probably been the cause of not a few of the discrepancies in results obtained with identical serums.

For the reasons given, then, I am in favor of the practice of titrating complement with a fixed dose of amboceptor and of varying the amount of complement according to its potency. The exact mode of doing this will be discussed below when the subject of dosage of complement is reached. Many workers (including myself) make a practice of doing a preliminary approximate titration of each guinea-pig's serum, and rejecting any which are decidedly below standard. At the same time the control tube is set up for detecting any guinea-pig's serum which contains an excess of natural antisheep hemolysin. This

5. Ottenberg: Correct and Incorrect Methods of Performing the "Daily Titrations" for the Wassermann Reaction and Other Forms of Complement Fixation, Jour. Immunol., to be published.

6. Kromayer and Trinchese: Med. Klin., 1912, viii, 404.

tube contains merely cell suspension and 0.1 c.c. of complement. It should show practically no laking.

VARIATIONS IN THE "FIXABILITY" OF GUINEA-PIGS' COMPLEMENT

In the discussion thus far it has been taken for granted that the complements of different guinea-pigs are equally amenable to fixation. But, as Browning and McKenzie have shown, this is by no means the case. They found that if several complements of equal hemolytic power were tested under the same conditions, sometimes one and one-half times as much of one would be fixed as of another. And repeating the proof of this fact in a different way, I have repeatedly found that of four or five complements of equal hemolytic titer tested simultaneously with alcoholic antigen extracts, the one would give complete inhibition with the positive serum in a dilution of only one-fourth, while another would give equal results with the positive serum in a dilution of one-eighth, and still others with the serum in dilutions of one-twelfth or one-sixteenth.

There is no important fact in the whole subject of complement fixation that has been so neglected as this mysterious phenomenon; indeed most workers seem completely to ignore its existence. And yet probably it is at the bottom of many contradictory reports from different laboratories. It is true that by using the mixed complements of a large number of guinea-pigs one may strike a reasonable average of fixability. And this is one of the unrecognized reasons why reports from large laboratories where many tests are made are more reliable than from small laboratories where few are made, even though the workers be equally competent. But even the average of several specimens is bound to vary unless the number which goes to make the average is impracticably large.

In the future, if the Wassermann reaction is to become standardized, as for instance the production of diphtheria antitoxin has become standardized (and the former task should be no more difficult than the latter), then, among other things, some simple method of testing the fixability of guinea-pigs' complement in the preliminary titration will have to be introduced so that specimens of inferior fixability may be rejected. As the result of experiments now under way I hope in the near future to be able to suggest a method. At present the only way to avoid this source of error is to use mixed complements of a large number of guinea-pigs; four or five should always be used and if possible more.

Before discussing the dosage of complement for the reaction it will be necessary to take up certain considerations connected with the preparation of the sheep cell emulsion and amboceptor, the mode of incubation, and the anticomplementary effects of normal serum and of antigen.

SHEEP CELL SUSPENSION

The preparation of the sheep cell suspension has undergone but one modification since Wassermann's work and that is a modification which a great majority of workers have adopted. However, there are some workers, as for instance, Citron, who still adhere to the original method.

Wassermann used 1 c.c. of a suspension, which represented five volumes of the whole blood of the sheep diluted with ninety-five volumes of saline (the cells of course having in the meanwhile been carefully washed at least three times by centrifugalization). On account of the fact that various sheep do not agree in the number of red cells per cubic centimeter of their blood, most subsequent workers have used an emulsion representing five parts of compact sedimented washed red blood cells added to ninety-five parts of saline.

This really represents approximately twice as strong a red cell suspension as the original, a fact which does not seem to have received much attention. However, nearly all workers now use 5 per cent. of the sediment as their standard, and it would seem especially important to adhere to this method in case the same sheep is bled repeatedly and hence gradually becomes anemic. In measuring the sediment it is far preferable to measure in a graduated centrifuge tube, reading the upper level of the cell sediment at the end of a definite length of time (ten minutes) in a centrifuge of a definite number of revolutions per minute (from 4,000 to 6,000), rather than to depend on measuring the compact sediment with a graduated pipet.

When defibrinated blood is used (as it is by nearly all workers) it is important that the cell suspension contain no small clots. These contain a great number of red cells and by absorbing an undue share of amboceptor interfere with hemolysis in the particular tubes that they finally reach. They are removed by filtering the defibrinated blood through gauze or by very careful decantation.

The sheep red cells, as well as the complement, represent a variable factor, since it has been shown (Ottenberg and Hopkins[7]) that individual sheep vary considerably in their resistance to hemolysis. This factor is very hard to make allowance for, and probably the best method is to keep one or two sheep in the laboratory and use the blood of the same animal during as long a period of time as possible. However, the range of variation is not nearly so great as that of complement and probably plays a far smaller rôle in the irregularities in the results of the reaction.

THE TECHNIC OF AMBOCEPTOR TITRATIONS

The absolute titer of the particular amboceptor used is practically a matter of indifference (the term amboceptor will be used here for immune sheep red cell hemolytic serum). It can also be accepted as a fact that after the first few days the potency of amboceptor, if properly preserved, remains practically constant over long periods of time.

In determining the strength of a new amboceptor there are a number of apparently trivial points of technic which, however, may make a considerable difference in the result. The first of these is the element of time. Hemolysis is a reaction which progresses gradually and which is not completed at any one definite time. Therefore, the period of time taken at the end of which the degree of hemolysis is read is to a certain extent arbitrary. Wassermann made the readings in his titrations at the end of one hour in the air thermostat because at the end of that time hemolysis is almost complete. Thomsen, Boas, and others, however, because hemolysis is not absolutely complete at one hour, use a two-hour period. Stern used one and a half hours. Other workers in order to save time do their readings after a half hour (Sormani) or fifteen minutes (Field, Kaliski, and others).

7. Ottenberg and Hopkins: Proc. Soc. Exper. Biol. and Med., June, 1914.

In practice it makes very little difference which particular period is adhered to so long as the same period is always adhered to and so long as the same period is adhered to both in titrations and in the actual performance of the tests. But for purposes of standardizing the reaction it would seem highly desirable that workers agree on some uniform period. The original one-hour period of Wassermann seems rather long on account of the delay in practical work and in view of the fact that over 90 per cent. of hemolysis takes place within the first half hour. The fifteen-minute period is probably too short to allow for slight differences in the speed with which the mixtures assume the temperature of the thermostat due to variations in the thickness of glassware, etc.

Most authors fail to state whether their incubations are in air thermostat or in water bath, yet this is a very essential point, because, owing to the greater speed with which the contents of the tubes become warmed in the water bath, thirty minutes in the water gives about the same result as from forty-five to sixty minutes in the air thermostat. The water bath is preferable because the temperature of the air incubator changes every time its door is opened.

When a very long period is taken, such as two or more hours, another factor plays a considerable rôle, namely, the dissociation of amboceptor. Muir[8] has shown that a certain proportion of amboceptor can be dissociated from the substance of cells on which it has already acted and can then lake additional cells. For these reasons I prefer the half-hour period of incubation in the water bath, which is practically equivalent in result to one hour in the air thermostat. It is greatly to be desired that on this and other similar and more or less arbitrary points of technic some official standards be set by some of the representative organizations of serologists.

Next to the time factor, the manner in which the ingredients are mixed in the titration plays a considerable rôle. This is particularly true if a short time incubation is used. If cells are mixed with amboceptor for ten or fifteen minutes before the complement is added, they absorb the amboceptor and are sensitized and after the addition of complement hemolysis takes place much more rapidly than if the three ingredients are added simultaneously. The advantage of the longer periods of incubation is that (due possibly to dissociation) irregularities due to such factors as these are more or less equalized. Which of the two methods is used (preliminary sensitization of the cells or immediate incubation after mixing all three ingredients) is probably not of great moment. But it is important that the same method should be uniformly used throughout by each worker, a thing which does not seem by any means to be the case, as there are some workers who after titrating by one method use the other method in the actual tests.

A necessary detail which hardly needs to be mentioned to experienced workers, but which, nevertheless, is sometimes neglected, is immediate mixing of the reagents; for instance, if cells are added little by little to amboceptor the result is less laking than if all the cells are added at once, probably because, due to the great avidity of cells for amboceptor, the first cells added absorb more than their share of amboceptor. Likewise if a mixture is made and not shaken at once an uneven sensitization and imperfect laking occurs.

A factor of importance, of course, in determining the unit of amboceptor is the amount of complement used in the determination. Wassermann and the majority of workers following him use 0.1 c.c. of the mixed complement of several guinea-pigs. Some workers, as, for instance, MacIntosh and Fildes,

8. Muir: Studies in Immunity, 1909.

Walker, and Kolmer, use 0.05 c.c. Oluf Thomsen uses 0.08 c.c. of complement, explaining that it is an excess. While 0.1 c.c. of complement does not represent the largest possible excess of complement, nevertheless for the sake of uniformity it had probably better be adhered to. If the amboceptor unit as determined with a very large excess of complement were an absolutely constant and standard thing, it would probably be better to use such a Bordet unit. But (as was found in investigations of the board of health committee referred to above) this is not the case, and even with a great excess of complement, on different days, when the complement is obtained from different guinea-pigs, the amboceptor unit shows slight variation. Furthermore, when one uses amounts of complement decidedly above 0.1 c.c. the natural antisheep hemolysin of guinea-pigs often begins to interfere with the titration. It therefore seems best to titrate the minimal hemolytic dose of amboceptor with 0.1 c.c. of complement on a number of successive days and to take as the amboceptor unit the average of the minimal hemolytic amounts. If this is done, it is probable that workers in different laboratories can at least work with fairly uniform amounts of amboceptor and red cells.

THE DOSE OF COMPLEMENT

The factor which more than any other determines the accuracy of the reaction is the dose of complement used, and yet this is a factor on which different workers vary from each other perhaps more than in any other. Wassermann, followed by Citron[9] and a good many other workers, used a fixed dose of complement, namely, 0.1 c.c., the amboceptor and the antigen dose being adjusted so that this amount of complement would represent a sufficient excess to prevent any nonspecific fixations. Subsequent workers have modified the amount or the principle of determining the amount of complement with the general idea that the smallest amount compatible with safety should be used.

In reasoning on the subject certain factors which interfere with the hemolytic action of complement must be recognized. Practically all the substances used as antigens, if taken in a large enough dose, interfere with the action of complement. Likewise, human serum, whether normal or syphilitic, interferes to some degree with the action of complement, and this anticomplementary power of serum is very variable for different individuals, and furthermore, it depends on the age and condition of the serum. Beside this it is possible that some normal serums have a very small but appreciable power of reacting with antigen in a nonspecific way. It was to prevent false positive results due to these factors that Wassermann established his standard of a hemolytic system of double strength (two units of amboceptor) and the dose of antigen the double of which should not of itself at all inhibit the action of the hemolytic system used.

It has appeared to most of the subsequent workers that this system provides a somewhat larger safety zone than is needed, and the general

9. Citron: Immunity, translated by A. L. Garbat, 1914.

effort has been to find some method of making the reaction more delicate by diminishing the amount of complement, while at the same time making sufficient allowance for the nonspecific anticomplementary factors mentioned. As a matter of fact, the great majority of workers now use an amount of complement which is considerably less, generally from one half to three fifths of the amount prescribed by Wassermann.

Wassermann's quantity, 0.1 c.c., is an actual excess and really represents not merely two units of complement with two units of amboceptor, but probably three or more units of complement with two units of amboceptor. This can readily be shown by retitrating the amboceptor unit which has already been determined with 0.1 c.c. of complement, but doing the titration with smaller amounts of complement. It is found that a considerably smaller amount of complement, for instance sometimes 0.06 c.c., will give the same amboceptor unit. Kromayer and Trinchese[10] pointed this out in 1912. They showed that if an arbitrary (not minimal) amount of complement is used with descending amounts of amboceptor, the least dose of amboceptor that gives complete laking will be found to do so with a still smaller dose (up to 30 per cent. less) of complement. This was confirmed by M. Stern[11] in 1914.

I have often repeated the experiment in the form given below. The amboceptor unit was first determined with 0.1 c.c. complement as follows:

I. Complement 1 to 10, 1 c.c.					
Amboceptor (No. 267, 1 to 200).....	0.15	0.2	0.24	0.28	0.0
Thirty-minute reading	c?*	c	c	c	

* The letter "c" signifies complete laking.

The amboceptor unit was thus found to be between 0.15 c.c. and 0.2 c.c. of the 1 to 200 dilution and was estimated as being 0.18 c.c. Now a titration of the identical complement with two of these amboceptor units, namely 0.36 c.c. was done as follows:

II. Amboceptor 0.36 c.c. (2 units)			
Complement 1 to 10.....	0.25	0.3	0.35
Thirty-minute reading		c	c

It was thus seen that with two amboceptor units the least amount of complement which would give complete laking was 0.3 c.c. of the 1 to 10 dilution. This might therefore be termed the complement unit with two units of amboceptor.

Now with two such units of complement, namely 0.6 of the 1 to 10 dilution, the amboceptor was again titrated and on comparing this with the first of the three titrations it is seen that the amboceptor unit obtained with 0.6 c.c. of complement is practically identical with the amboceptor unit obtained with 1 c.c. of the 1 to 10 complement.

III. Complement 1 to 10, 0.6 c.c. (2 units)				
Amboceptor (No. 267, 1 to 200)....	0.15	0.2	0.24	0.28
Thirty-minute reading	c?	c	c	c

10. Kromayer and Trinchese: Med. Klin., 1912, No. 10, p. 404, No. 41, p. 1670.
11. Stern, M.: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1914, **22,** 130.

Thus, if Wassermann tests were set up with 0.6 c.c. of the 1 to 10 dilution of this complement and two units (0.36 c.c. of the 1 to 200 dilution) of this particular amboceptor, the amount of complement would really represent two units of complement as determined with two units of amboceptor; and it is seen that 1 c.c. of 1 to 10 complement (namely, 0.1 c.c. complement) really represents three or more units. The author has found in practice, as have many other workers, that the smaller of these two amounts, namely, two units of complement and amboceptor, represents (with simple alcoholic extracts as antigens) an amply sufficient margin of safety, and gives far more delicate results than the larger, arbitrary dose of complement. The reason that led the majority of workers to use smaller amounts of complement was not, of course, any theoretical consideration such as these, but the practical experience that smaller doses were safe and gave better results. The question, however, by what criterion to decide just what dose to use has not been settled.

It is obvious that it would not be safe to use one unit of amboceptor with one unit of complement for the reason that this would make no allowance for the anticomplementary effects of antigen and of patients' serum. What is needed, of course, is one complete unit of complement (that is sufficient to produce complete laking) above and beyond that amount of complement which is neutralized by the nonspecific complement-binding powers of the anticomplementary agents present in the reaction.

Maslakowitz and Lieberman,[12] Browning and McKenzie[13] in 1909, and Thomsen[14] in 1910 made the first steps in this direction. Thomsen proposed to determine the free unit of complement by titrating the complement in the presence of antigen, and using in the tests that amount of complement which (with two units of amboceptor) just gives complete laking in the presence of the arbitrary amount of antigen used. In the serum control tube he used one unit (as determined with two units of amboceptor, but of course not in the presence of antigen). This method is correct in principle, but it does not go far enough, since it disregards the slight, but frequently present, anticomplementary effect of the patient's serum, with the result (as the author can testify from having tried the method extensively) that the patient's serum is occasionally found anticomplementary in the serum control tube, so that one cannot with safety report the result of the test.

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12. Maslakowitz and Liebman: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1913, p. 804.

13. Browning and McKenzie: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1909, **2**, 459.

14. Thomsen, Oluf: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, **7**, 389.

Margarete Stern[11] in 1914 apparently tried to get around this difficulty by increasing the hemolytic strength of the serum control tube, but not of the test proper. She kept the dose of complement constant (0.1 c.c.) and titrated the amboceptor in the presence of the antigen, using in the test that amount of amboceptor which caused complete laking in the presence of the antigen; in other words, using what in effect is equivalent to one free unit of amboceptor above the amount neutralized by the anticomplementary effect of the antigen. In the serum control tube she used not one but one and a half units of amboceptor. This seems a dangerous and deceptive practice, since the extra allowance of amboceptor for the anticomplementary effect of patient's serum is omitted precisely in that tube in which it is most important, the test itself.

The most ingenious and complete method of making accurate allowance for the anticomplementary factors in the test is the method of Browning and McKenzie,[13] 1909. These authors did a daily determination of the complement unit, using in this determination five units of amboceptor. They then set up a series of serum tubes containing one, two, three and four complement units for each serum. For the antigen control they likewise set up, with their arbitrary dose of cholesterinated beef heart antigen, tubes containing one, two, three and four units of complement. For the test itself they used a series of tubes containing 5, 7, 10, 15, 20, 30 and 40 units of complement. They thus determined simultaneously the anticomplementary value as measured in units of complement of the antigen and of each serum tested. They require in order that a serum be regarded as positive that it give inhibition with at least five complement units over and above the number of complement units inhibited by the serum itself and by the antigen. This system surmounts many of the obstacles which are encountered, but the use of the enormous amounts of complement in the last two or three tubes of the test (namely, twenty, thirty and forty units) is so wasteful as to seem impracticable, and as a matter of fact is seldom necessary.

Indeed, the present author's trial of this method demonstrated that in order to detect many positive serums which were detected by other methods (Thomas and Ivy, Citron, Walker and Swift) it was necessary to set up tests not with seven, ten, fifteen, etc., units of complements, but with three, five, seven, etc., units (titrated, of course, after the directions of Browning and McKenzie). In other words, Browning and McKenzie's stipulation that in order to be regarded as positive a serum must fix five or more units is found to be far too exacting. The fixation of two or more units of complement can (with cholesterinated beef heart extracts) be safely regarded as specific.

With the slight changes suggested the method of Browning and McKenzie deserves very careful trial. One of its great advantages is that with it serums which are in themselves to a considerable degree anticomplementary can be tested without difficulty, as the serum control tubes represent a titration of this anticomplementary property and one

simply has to subtract the number of complement units found inhibited in the serum control in order to make allowance for the anticomplementary property of the serum.

One of the most satisfactory methods of determining the complement doses according to the principles laid down by Maslakowitz and Lieberman[12] and by Thomsen,[14] is the method proposed by Thomas and Ivy* in 1914. It follows the method of Thomsen fairly closely, but it has the advantage of making an allowance for the anticomplementary effect of normal serum, as is done in Browning and McKenzie's method, and it might be called a combination of Thomsen's and Browning and McKenzie's methods.

It consists of using in the test the minimal amount of complement which gives complete laking when titrated in the presence of the arbitrary dose of antigen and the amount of negative serum used in the test. The negative serum for this titration is pooled negative serum from several patients. Before the amboceptor and cells are added for the titration, the complement, serum and antigen are incubated as for the test proper, so that the anticomplementary effects of antigen and of serum (whether normal or syphilitic) are imitated under exactly the same conditions as those under which they exert their influence in the test. The dose of amboceptor used represents approximately two or three units. It is necessary to pick out such negative serums as contain no natural antisheep amboceptor, otherwise the complement titration is illusory.[15] In the serum control tube the amount of complement used is that which gives complete laking in an exactly similar titration done in the presence of pooled negative serum, but not of antigen. The amount of complement in each tube, therefore, represents exactly one free unit above the amount neutralized by the anticomplementary properties of pooled negative serum in one case, and of negative serum and antigen in the other.

I have used this system extensively and believe that it represents the most delicate and accurate way of determining the safe minimal dose of complement to use. In practice, however, it was found not safe to use the absolutely smallest amount of complement which just gave complete laking, for the reason that human serums, even when fairly fresh and recently inactivated, vary slightly in their anticomplementary property. Hence, if one uses the minimal amount which in the titration gives complete laking, then a serum which has slightly more anticomplementary property than the pooled negative serum used in the titration will give an unsatisfactory result, due to the serum control tube being incompletely laked.

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* Thomas, B. R., and Ivy, R. H.: Applied Immunology, 1915.

15. Ottenberg and Frazier: Jour. Infect. Dis., 1915, xvi, 119.

For example, if the three normal serums entering into the pool have anticomplementary values which we will represent by the figures, 5, 3, and 1, then the anticomplementary value of the pool would be 3 (the average) and the test done with the serum whose anticomplementary value was 5 will not lake completely in the serum control tube or the main tube. For this reason the author makes a practice of using the next dose above that which gives complete laking both in the titration with pooled negative serum and in that with pooled negative serum plus antigen. As the tubes in the titration differ by only 0.005 c.c. of complement, this represents only a very minute amount above the one free unit.

It is generally agreed that with cholesterinated antigen a stronger hemolytic system is required than with simple alcoholic extracts, but there is the same lack of uniformity as to the method of determining the strength of this hemolytic system as there is among those who work with simple alcoholic antigens. Thus Sachs,[16] according to Schlossberger, uses the fixed dose, 0.1 c.c. of guinea-pig complement, but uses four units of amboceptor, and, as a further precaution, uses half the amount of patient's serum employed in the original Wassermann reaction. Field[17] specifies from six to eight units of amboceptor with from three to four units of complement, although he does not describe exactly how these units are determined. Walker[18] and Swift use two units of amboceptor and two units of complement, the unit of complement being determined with a single unit of amboceptor, so that the amount of complement is generally about 0.1 c.c., and the actual strength of the hemolytic system is approximately fourfold. The work of Browning and McKenzie described above shows that the same principle of subtracting the anticomplementary effect of antigen and of patient's serum from the total amount of complement fixed, as is used in Thomsen's and in Thomas and Ivy's methods, with alcoholic antigen can be applied to the cholesterinated antigen. But the amount of free complement which they specify must be fixed (five units) over and above these anticomplementary effects in order that the reaction may be regarded as a positive one is of course greater than with the alcoholic extracts, and, according to my experience, as mentioned above, is too great.

PATIENT'S SERUM

The one element in the reaction the handling of which has undergone the least modification is the patient's serum. The amount prescribed by Wassermann, 0.2 c.c., as being sufficient to give positive results and yet safe within the range of the amount which may give a nonspecific result has been adhered to by the great majority of workers.

16. Sachs: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1913, **20,** 115.
17. Field: The Archives Int. Med., 1914, **13,** 790; Jour. Am. Med. Assn., 1914, **62,** 1620.
18. Walker, I. C.: The Archives Int. Med., 1914, **11,** 563.

Sachs, according to Schlossberger (1913), Browning and MacKenzie (1909), Thomas and Ivy (1914) and some others used a half of the usual dose, namely, 0.1 c.c. Schlossberger acknowledges that the larger dose increases the sensitiveness of the reaction and is safe provided one uses a slightly more active hemolytic system. It is interesting to note that all the workers who use the smaller dose of patient's serum use relatively very large doses of antigen extract.

On the other hand, there have been a number of attempts, such as those of Kromayer and Trinchese[9] (1912), Ledermann[19] (1913) and of Thiele and Embleton,[20] to increase the delicacy of the reaction by increasing the proportion of patient's serum. Thiele and Embleton, who use up to five times the usual quantity, attempt to compensate for this by using a relatively much smaller amount of antigen than other workers, and although the general opinion of other workers seems to be that this method is unsafe, it does not appear to have been tried by others as it deserves to be. Field, in addition to using the regular amount, uses a tube containing double the amount of serum, but he regards a positive result obtained with this amount in the presence of a negative result with the regular amount as being merely a "suspicious" reaction.

With spinal fluids the use of far greater amounts has been found to be safe and to detect reactions which would otherwise be lost. The method introduced by Hauptmann* of using graded amounts up to 1 c.c. is now generally followed. Some workers even carry the dosage up to 2 c.c., and as long as the control is satisfactory this seems justifiable.

INACTIVATION

Practically all workers inactivate the patient's serum by heating in a water bath for a half hour at 56 C. This is not done, however, with spinal fluids, unless they are several days old before being tested. Inactivation of course has for its purpose not only the removal of the unknown additional amount of complement in the patient's serum, but the destruction of the anticomplementary effect of some serums, the latter being the more important.

The observation of Seligman and Pincus[21] that the use of active patient's serum leads to a certain proportion of false positive reactions has received general acceptance. Only Graetz[22] (1913) denied this and believed that with proper technic it was possible to get perfectly reliable results with active serum. Noguchi showed that the false positive results with active serum were due to certain impurities, probably lipoid-protein combinations in the antigen, which were removed by his method of acetone precipitation of the alcoholic extracts, and he recommended that with his acteone insoluble lipoids active serum be used. In spite of this and of the undoubted fact that heating destroys some of the positive reacting substances, practically all serologists at present agree that inactivation is necessary to avoid nonspecific results.

THE SERUM CONTROL TUBE

The amount of patient's serum to be used in the serum control tube without antigen has undergone but one modification, and this is one which is very widely used.

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* Hauptmann and Hössli: München. med. Wchnschr., 1910, **57,** 1581. Hauptmann: Deutsch. Ztschr. f. Nervenh., 1911, **42,** 240.

19. Ledermann: Med. Klin., 1913, No. 50, p. 2071.

20. Thiele and Embleton: Ztschr. f. Immunitätsforsch. u. exper. Therap., **16,** No. 4, p. 430.

21. Seligman and Pincus: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, **5,** 377 .

22. Graetz: Med. Klin., 1913, **9,** No. 45, p. 1858; ibid., No. 46, p. 1898.

Wassermann himself prescribed that a control tube containing double the amount of patient's serum used in the main tube should not give inhibition, but experience has shown that he was more cautious than was necessary, and a great many workers now use the same amount of serum in the serum control as in the main tube. Some, such as Sachs (according to Schlossberger), Walker and Swift, and Bruck,[23] adhere to the double dose.

The use of the double dose of serum in the control tube superficially appears to be an additional factor of safety. In reality this is not the case. It is true that if the patient's serum possesses a certain amount of anticomplementary property, such as might lead to a false positive result, this is accentuated and hence more easily detected if the double dose is used. On the other hand, if the patient's serum happens to contain a considerable amount of antisheep hemolysin, the control will contain double as much as the main tube, and the more complete and rapid laking in the control tube can falsely give the appearance of a positive result. The identical dose of serum in the control as in the main tube certainly gives a more accurate picture of the effect the serum is having in the main tube, and this is the real object of a control tube. For these reasons I believe that the dose of serum in the control tube should be the same as that in the main tube.

THE DOSAGE OF COMPLEMENT IN THE SERUM CONTROL TUBE

To a worker who is accustomed to following the original Wassermann technic rigorously the use of different doses of complement in the serum control tube and in the main tube, as in the methods of Thomsen, of Boas, and of Thomas and Ivy, or of a different dose of amboceptor in the two tubes, as in the method of Stern, at first seems to deviate from the fundamental principles of the test. But, as a matter of fact, it does not, since it merely makes allowance for the different anticomplementary factors present in the two tubes. In fact, this method really offers a safer result than the original one of equal doses of hemolytic components in the control and main tubes. For in the original method in order to be sure to have enough complement in the main tube to overcome any anticomplementary property of the antigen, the dose of complement in the control tube, being the same as in the main tube, is considerably in excess. But this excess of complement in the control tube is enough to mask a certain degree of anticomplementary effect of the serum; and false positive results due to the addition of anticomplementary effects in the main tube are therefore harder to detect.

Thus, suppose (an actual instance) the titration shows that for the

23. Bruck: Serodiagnosis of Syphilis, 1909.

original Wassermann system the required dose of complement (two units) is 0.1 c.c., and the amount needed for a system such as Thomas and Ivy's in the tube containing both antigen and serum is 0.09 c.c., and in the control tube with serum alone 0.06 c.c. In the original system, of course, the control tube with serum alone gets the same dose of complement as the main tube, 0.1 c.c. But this excess may be enough to mask the anticomplementary effect of some particular serum and nevertheless this anticomplementary effect, added to the slight anticomplementary effect of the antigen in the main tube, may be enough to give falsely a weak positive result.

To make this clearer, consider the above instance; the unit of complement was found to be 0.05 c.c., the unit of complement as determined in the presence of pooled negative serum 0.06 c.c., the unit as determined in the presence of serum and antigen 0.09 c.c. From these figures it is clear that the pooled negative serum has an anticomplementary value of 0.01 c.c., and the antigen an anticomplementary value of 0.03 c.c. Now it is clear from this that in the original Wassermann, in which the identical amount of complement, 0.1 c.c., is used in both main and control tubes, in the average case in the main tube there is 0.06 c.c. free complement, and in the control tube 0.09 c.c. But suppose that among the serums to be examined on a particular day there was one whose anticomplementary value was as high as 0.05 c.c. of complement, then in the original Wassermann this would still leave in the control tube 0.5 c.c. of complement, sufficient to give complement laking in the control tube, while in the main tube this anticomplementary effect added to the slight (0.03 c.c.) anticomplementary effect of the antigen would use up 0.08 c.c. of complement and leave only 0.02 c.c., in other words, would give the appearance of partial inhibition or a false positive reaction, since the control tube would be completely laked. On the other hand, if the tests were set up so that an exact unit above the average anticomplementary effect of human serum was used in the control tube, rather than the huge excess of the original Wassermann, the anticomplementary property of this serum would be detected readily and a false positive result would not be reported.

This is undoubtedly one of the reasons why all workers refuse to attribute much diagnostic value to weak inhibitions with the original Wassermann system. It is likely that the practice of more accurate adjustment of the complement dose in the serum control tube will make weak inhibitions more significant. It is the particular advantage of the system of Browning and McKenzie that it makes accurate allowance for the anticomplementary effect of each serum tested.

In the original technic of Wassermann the attempt was made to avoid this source of error by the use of the double quantity of patient's serum in the serum control tube. And for those who adhere to the original method in

this regard, using the same strength of hemolytic system in the serum control tube as in the main tube, this is probably the safer procedure. Unfortunately, however, in case the patient's serum contains any natural antisheep amboceptor, the control tube will contain twice as much as the main tube; and this, if one depended on the natural antisheep amboceptor, could easily give one a false weak positive result. Thus, for instance, if the ordinary dose of serum (0.2 c.c.) contained not quite enough antisheep amboceptor to produce complete laking, the control tube containing twice as large a dose of serum would contain more than enough to produce laking and one would get the appearance of partial inhibition in the main tube with complete laking in the control tube. Therefore, if the test is done with double serum control, one must invariably add amboceptor and disregard any appearance of a positive reaction obtained with natural amboceptor alone. To do this, however, as will be shown below, causes one of necessity to miss a certain small proportion of weakly positive reactions, which become negative due to the excess of natural amboceptor present. It is possible, of course, to avoid missing these by repeating all the tests that contain much natural amboceptor after its absorption or by subjecting all serums to absorption in the first place. This subject will be further discussed below under the heading of natural antisheep amboceptor.

THE MANAGEMENT OF NATURAL ANTISHEEP HEMOLYSIN IN THE PATIENT'S SERUM

Many authors have referred to the frequent occurrence of natural antisheep amboceptor in appreciable amounts in human serum.[24] The well-known modification of Bauer, as well as that of Hecht and Weinberg, take advantage of this; and the substitutes for the Wassermann method, such as that of Noguchi, are devised chiefly to eliminate the error caused by it. The occasional occurrence of a great excess of such natural amboceptor is also well recognized. Thus Ottenberg and Frazier found in 2,158 tests that 955 serums (44 per cent.) had an appreciable amount of natural amboceptor, and that 476 (21 per cent.) had sufficient to produce complete laking without further addition of amboceptor.

In obtaining these figures the complement was always titrated and the amount used represented two complement units, as discussed above under the head, dose of complement, and was generally about 0.06 or 0.08 c.c. Those samples of complement which were below the average were rejected and not used, while those which were above the average in activity were used in a smaller dose so as to equal the average. If all the human serums had been tested with the arbitrary amount (0.1 c.c.) of guinea-pig complement used in the original Wassermann reaction, it would have been found that a far larger proportion show natural amboceptor, as smaller amounts of natural amboceptor are brought out by greater amounts of complement. (In fact with larger amounts of complement over 90 per cent. of human serums are found to contain some antisheep amboceptor.) Thus when Wassermann tests are done using a fixed amount of guinea-pig serum as complement, as is done by

24. Wassermann, Neisser, Bruck and Schucht: Ztschr. f. Hyg. u. Infectionskrankh., 1906, **55**, 451. Jacobaeus: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1911, **10**, 321. Rossi: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1911, **8**, 615. Dexter and Cummer: THE ARCHIVES INT. MED., 1912, **9**, 605. Olmstead: Med. Rec., New York, 1914, **85**, 341.

Citron, Stern, Sachs, and many others, the possibilities of error due to the presence of natural amboceptor are really much greater than when the amount of complement is varied according to its strength.

Although some workers pay no attention to natural amboceptor, a perusal of the literature and my own experience leave no doubt that if the presence of natural amboceptor is disregarded a certain small percentage of false negative reactions is obtained. Some authors have undoubtedly exaggerated the proportion of such cases. Nevertheless, it is obvious that the occasional coincidence of a large excess of natural amboceptor with a small amount of so-called syphilitic antibody will inevitably lead to this error. In spite of the many methods which have been proposed to obviate this difficulty there is none entirely satisfactory. The absorption methods are accurate but cumbersome, and sometimes render serums anticomplementary (Sachs' phenomenon). Some observers make a preliminary test, often called the Bauer control tube, to determine the natural amboceptor, and then add immune amboceptor only to those serums that need it. Others, like Graetz[25] and like Snow and Cooper,[26] make a routine practice of absorbing the natural amboceptor from all serums. Browning and McKenzie[13] use ox cells instead of sheep cells, because natural antiox hemolysin is of less frequent occurrence in human blood than antisheep hemolysin.

In general, the error due to natural hemolysin is greatest in those systems which use relatively large doses of complement; and in the systems which (like those of Thomsen[14] and Thomas and Ivy*) reduce the complement to one free unit this error is reduced to a minimum.

The method which I have adopted for use with the system containing two hemolytic units of complement originated with Kaliski and is similar to the Bauer method, but simpler. It consists of the addition of unsensitized sheep cells to all the tubes and incubation for ten minutes in the water bath at 37 C. At the end of this time those tests which contain a considerable excess of natural amboceptor show complete laking in the control tubes, and to these tests no amboceptor is added. To all the other tests the usual amount of two units of amboceptor is then added, and all the tests are incubated for the full period. Thus, the addition of still more amboceptor to those tests that already contain an excess is avoided.

It must be borne in mind that even with this method those rather exceptional serums that have a very great excess of natural amboceptor combined with weak Wassermann reacting bodies may still give false negative results. These serums (recognized by rapid laking in all the tubes) are retested after absorption of the natural amboceptor. How

25. Graetz: Med. Klin., 1913, **9**, 1858, 1898.
26. Snow and Cooper: Am. Jour. Med. Sc., 1916, **152**, 185.
* Thomas and Ivy: "Applied Anatomy," 1915.

valuable absorption has proved in these cases may be seen from the fact that in the last eight months of 144 negative reacting cases which were retested after absorption of a considerable excess (two or more units) of natural hemolysin, thirty-six cases became definitely positive. Of these, seventeen became weakly positive (plus or double plus) and nineteen became strongly positive (triple plus or quadruple plus). All of these were cases in which there were good clinical grounds for suspecting syphilis.

If the reliability of the reaction is to be increased it seems to me that all those methods of complement fixation which depend exclusively on the natural hemolysin of human serum, as well as those like the Hecht-Weinberg reaction, which depend also on the natural complement, should be rejected. Ingenious as these methods are, they should be regarded as tours de force which tend away from, rather than toward, accuracy and reliability.

PRIMARY INCUBATION

Bordet and Gengou, Wassermann, and other early workers in complement fixation experimented with various periods of time and various temperatures for the incubation during which the fixation of complement occurred. It was found that the temperature does not play a very great rôle, but that fixation occurs somewhat better at temperatures approximating 37.5 C. than at other temperatures. It was found also that fixation is not a reaction which occurs suddenly, but one that occurs progressively and that in general the longer the time the more complete the fixation of complement. However, as it was found that at 37.5 C. complement deteriorated after several hours; and as most of the fixation possible occurred in the first hour, Wassermann established the rule of doing complement fixations at 37.5 C. for one hour, and until recently this was adhered to by most workers.

On account of the fact that fixation goes on almost as well at lower temperatures (from 8 to 10 C.) as at body heat, and that at these temperatures the deterioration of complement in longer periods than one hour is exceedingly slow, Jacobsthal,[27] 1910, advocated doing the preliminary incubation in the icebox at from 8 to 10 C. for longer periods (from three to four hours). This, the so-called icebox method, has been tried out by a great many other workers (Altman,[28] 1913, Leredde and Rubinstein,[29] 1914), and has been found to give results decidedly superior to the one-hour incubation at 37.5 C., and to give no false positive results whatever. Very much longer periods, such as from twelve to eighteen hours in the icebox, have been found unsatisfactory.

In many hundreds of tests done in parallel with icebox and with thermostat (37.5 C.) incubation, I have been able to confirm this, and I

27. Jacobsthal: Deutsch. Med. Wchnschr., 1913, **39,** 1337; ibid., No. 29, p. 1435.
28. Altmann: Arch. f. Dermat. u. Syph., 1913, **116,** 871.
29. Leredde and Rubinstein: Bull. Soc. franç. de dermat. et de syph., 1913, No. 2, p. 93.

Year	Author	Cell Emulsion	Amboceptor Dose	Complement Dose	Patient's Serum	Serum Control	Antigen Dose	Antigen Control
1906	Wassermann...	1 c.c., 5% of full blood	2½ units (at 1 hr. with 0.1 complement)	Fixed dose 0.1.....	0.2	0.4	½ amt. not of itself anticomplementary (watery, later alcoholic extract)	Double dose
1909	Bruck.....	1 c.c., 5% of full blood	3-4 units.....	Fixed dose 0.05.....	0.2	0.4	½ amt. not itself anticomplementary (alcoholic extract syphilitic liver)	Double dose
1909	Citron.....	1 c.c., 5% of full blood	2 units (at 1 hr. 0.1 complement)	Fixed dose 0.1.....	0.2 and 0.1	0.2	½ amt. not itself anticomplementary (alcoholic extract normal organs)	Double dose
1913	Sachs (Schlossberger)	1 c.c., 8% of sediment	4 units (unit at 1 hr. 0.1 complement)	Fixed dose 0.1.....	0.1	0.2	½ amt. not itself anticomplementary (cholesterinated beef heart)	Double dose
	Noguchi.....	1 c.c., 5% of sediment	2 units (unit at 1 hr. excess complement)	2 units (unit = smallest amt. that lakes in 2 hr. unit of amboceptor.)	0.2	0.2	½ amt. not itself anticomplementary (acetone insoluble lipoids) (0.2 of 0.3 % emul. of lip.)	Double dose
1914	Thiele and Embleton	0.4 c.c., 5% of sediment	2 units.....	1¼ units (0.2 by calculation, but not stated clearly	0.2 and 1.0	0.2 and 1.0	About 0.2 c.c. of 1/10 dil. acetone insol. lip. (said to be 50 to 100 units). Acetone insol.	Antigen shows no anticomplement
1914	Walker and Swift	1 c.c., 5% of sediment	2 units (unit at 1 hr. 0.05 complement air incubation)	2 units (generally 0.08-0.1) (unit 1 hr. with 1 unit of amboceptor)	0.2	0.4	Not over ¼ amt. that is completely anticomplementary (cholesterinated human and beefheart)	Single dose
1914	Field.....	1 c.c., 5% of sediment	6-8 units.....	3-4 units	0.2	0.2	⅛ anticomplementary dose (= dose shows beginning anticomplementary effect)	.....
1910	Browning and McKenzie	2 c.c., 5% of ox blood (5% of whole blood)	5 units (unit at 1 hr. with 0.2 complement)	1, 2, 3, 4 units for antigen and serum control (a and b) 7, 10, 15, 20, 30, 40, units for test (c) (unit 1 hr. with 5 amboceptor units, unit = 0.1-0.2)	0.1	0.1	Arbitrary (very large) dose 1.2 c.c. 1/6 dil. cholesterin beef heart	Single dose
1910	Oluf Thomsen.	5% of sediment	2 units (unit at 2 hr. with 0.08 complement, an excess)	1 unit for control tube 0.04. 1 unit above. amt. neutralized by antigen for main tubes (0.08) (unit 2 hr. with 2 units of amboceptor)	0.2, 0.1, 0.05, etc.	0.2	Arbitrary fixed dose (0.2) of alcoholic heart extract	Single dose
1912	Harold Boas...	5% of sediment	2½ units (unit at 2 hr., 0.1 complement)	Same as Thomsen.....	0.2, 0.1, 0.05, etc.	0.2	Like Thomsen	Single dose (anticomp. in double)
1911	Sormani.....	5% of sediment	8-12 units so that laking is finished in ½ hour	Same as Thomsen except ½ hr. laking and excess of amboceptor	0.2	0.2	Arbitrary dose (0.25-0.3) alcoholic heart extract	Single dose
1914	Margarete Stern	5% of sediment	1½ units in serum control; 1 unit above amt. used up by antigen in main tube (1½ hr. with 0.1 complement)	Fixed dose 0.1.....	0.2	0.2	Arbitrary dose (0.3 alcoholic extract), 5 different antigens for each test	Single dose
1914	Thomas and Ivy	5% of sediment	2-3 units (arbitrary fixed dose — 1 c.c. 1/1000, whose titer is 1/2000 or 1/3000)	1 unit above amt. neutralized in prelim. incubation by antigen and pooled serum (generally about 0.05). For serum control 1 unit above amt. neutralized by serum	0.1	0.1	Arbitrary dose (0.2 alcoholic extract of syphilitic liver)	Single dose
1916	New York Department of Health	5% of sediment (cells washed 6 times)	3 units (unit 1 hr. with 0.1 complement water bath)	Fixed dose 0.1.....	0.2	0.4 and 0.2	3-4 times the amt. used is not anticomplementary and ¼ amt. gives complete fixation with a +++ serum (almost always 1 c.c. of 1/25 dil. alcoholic extract guinea-pig heart)	1, 2, 3, 4 doses (prelim. incub. in icebox like tests)

* For purposes of comparison the quantities have been translated into terms of the original Wassermann quantities. In a few instances the failure of the authors to define their terms precisely has made this difficult and may have led to slight inaccuracies in the table. The New York Health Department method was demonstrated to the author by Mr. Koopman. The other methods are from the literature.

Incubation a. Prelim. b. Final	Quantitative Reading	Special Points	Natural Hemolysin	Mode of Titration
a. 1 hr. / 37.5 C. b. 1 hr. \ air (or water bath)	Degree of laking			Daily determination of amboceptor unit with 0.1 c.c. complement
a. 1 hr. } 37 C. b. 1 hr. }		Sensitizes cells 15 minutes...	Acknowledged as unavoidable source of error	Daily amboceptor titration with 0.05 c.c. complement
a. 1 hr. / 37 C. b. 1 hr. \ air	Tube 1 full doses, Tube 2 half doses ant. and serum. 1 and 2 complete ++++, 1 comp. 2 partial +++, 1 comp. 2 none ++, 1 partial 2 none +	Sensitizes cells		Daily amboceptor titration with 0.1 c.c. complement
a. 1¼ hr. / 37 C. b. 1-2 hr. \ air	Estimated by descending doses of antigen	Sensitized cells. Uses 4 doses antigen; as control uses 4 double doses; reads tube whose double antigen is completely laked		Daily amboceptor titration with 0.1 c.c. complement
a. 1 hr. } 37 C. b. 2 hr. } water bath read 4-6 hr. later room temp.	One extra tube with half dose serum. Readings after Citron scale (though both tubes get full dose antigen)			(Daily complement titration with one amboceptor unit)?
			Disregarded	Daily complement and amboceptor titrations ("simultaneous")
a. 1 hr. } 37 C. b. 1 hr. } air	Degree of laking	Uses always 3 antigens; positive result with any one is conclusive	"Bauer tube" with ½ dose serum. If completely laked may absorb	Daily complement titration with one unit amboceptor
	Six arbitrary doses antigen (similar to Sormani)			Daily complement titration (not described exactly)
a. 1½ hr. / 37 C. b. 1¼ hr. \ b. 1¼ hr. }	Read the number of complement units fixed above sum of units fixed by extract alone and serum alone. Positive only if 5 or more units ment units fixed by extract		Ox cells used because hemolysin for them is rarer	Daily titration of complement unit
a. 1 hr. } 37 C. b. 2 hr. } reads next day	Descending doses of serum (0.2, 0.1, 0.05; if needed, 0.025, 0.012, 0.006). Hemoglobin scale; reports positive only if over 50% inhibition	Anticomplementary serums tested by titrating amt. complement serum alone destroys and repeat test with this amt. added to all tubes		Daily titration of complement unit and of complement unit in presence of antigen dose
a. ¾ hr. / room ¾ hr. \ 37 C. b. 2 hr. reads next day	Like Thomsen			Like Thomsen
a. 1 hr. / 37 C. b. ½ hr.)	Descending antigen doses (smallest is least that gives + result with secondary syphilitic serum. Five tubes reads, 2/10, 4/10, 6/10, 8/10, 10/10 positive)			Like Thomsen
a. 1 hr. / 37 C. b. 1½ hr. \	None			Daily titration of amboceptor unit (with 0.1 c.c. complement) and of amboceptor unit in presence of dose of antigen
a. 1 hr. } 37 C. b. 1 hr. }	Increasing units of complement (2, 3, 4, or more). Units determined in separate titration are added to dose used in main tube. Thus 2 units in test means dose in main tube plus 1 bare unit			Daily titration of complement unit (with fixed dose amboceptor) and of complement unit in presence of antigen and normal serum together and of normal serum separately
a. 4 hr., ice box, 8 C. b. 1 hr. water bath, 37 C.	Tube 1 full dose, Tube 2 half dose serum, but both full dose antigen; readings on Citron scale	Separate amboceptor titration of each pig; rejects those that are below average. All tests done in duplicate	Receives no attention. Each guinea-pig is, however, tested for natural hemolysin and rejected if any is present	Daily determination of amboceptor unit with 0.1 c.c. complement 1 hr. in water bath 37 C.

believe that at least with the alcoholic antigens the icebox method gives by far the most delicate and accurate results. In experiments with weakly positive serums I have found that at very low temperatures (from 0 to 2 C.) fixation is not so good, but that at the temperature of the ordinary icebox (from 8 to 10 C.) fixation in a given time is almost as good as in the thermostat, and in the longer period (four hours) is much better. With cholesterinated antigens, however, the icebox method gives a considerable proportion of false positive reactions, and with that type of antigen, therefore, it should not be used. Swift, who is one of the principal advocates of cholesterinated antigen, stated recently his belief that with simple alcoholic extracts incubated in the icebox the results were practically identical with those obtained with cholesterinated antigen in the thermostat.

On the other hand, it is claimed by Altman[30] that there are some serums, particularly in primary syphilis, that give a better reaction at 37 C. For this reason, possibly, it is wise to combine the two methods (icebox and thermostat incubation) so that if there is any specific difference in the optimum temperature for fixation between serums, both kinds of serums can be detected. This is done by Kaliski, who uses two hours icebox, followed by a half hour thermostat, incubation.

THE ANTIGEN

The most disputed point in the whole technic of the Wassermann reaction has been the antigen, the question of which particular type of antigen is best, and how it should be used. It is practically impossible to come to any conclusion on this question from the perusal of the literature. Different workers have worked with such different technic that in most instances no direct comparison of their results is possible. Probably no agreement can be reached until a more standard and uniform technic is used in comparing the different antigens. The first point for us, therefore, is to try to establish the best mode of using antigen in general.

Considerations Concerning the Dosage of Antigen.— Since the Wassermann reaction is a quantitative reaction, naturally the prime question with any antigen is how to ascertain the correct amount of it to use in the test. In general, it is true of any antigen that the greater the amount one can use of it, the more delicate the test, but that the nearer one approaches the amount that begins of itself to become anticomplementary, the greater the danger of nonspecific fixations. There seems to be an exception to this rule in the as yet unconfirmed observation of L'Esperance and Coca,[31] that with the acetone insoluble lipoid

30. Altman: Dermat. Ztschr., 1912, **19**, 22; Arch. f. Dermat. Syph., 1912, **111**, 871.

31. L'Esperance and Coca: Jour. Immunol., 1916, **1**, 129.

fraction there is a prezone, that is to say, that when the tests are done in the particular way in which Coca does them, if the antigen is increased beyond a certain point, one fails to get a positive reaction with some serums.

In experimenting to determine the correct dose of antigen to use for Thomsen's or Thomas and Ivy's methods of doing the reaction, I have come across certain facts, which, while not suggesting a prezone, indicate that the larger dose of antigen does not necessarily give a positive reaction with a weaker reacting serum than does a smaller dose of antigen, in each instance the complement dose being so adjusted that no anticomplementary effect is to be feared from the antigen. It will be remembered that in these methods (those of Thomsen, Boas, Thomas and Ivy) the dose of antigen recommended was quite arbitrary and the complement dose was adjusted to the antigen in each case. It seemed to me in working with these methods that the arbitrary dose chosen might not be the optimum one. Experiments were therefore made with larger doses with the surprising result that when the complement dose was enlarged sufficiently to allow for the larger anticomplementary effect of the larger dose of antigen, complement fixation was not obtained with so high dilutions of a positive serum as when a smaller dose of antigen was used. The reverse experiment was therefore tried, namely, the use of smaller doses of antigen than the arbitrary ones usually given, and it was found that actually somewhat better results could be obtained with smaller doses, namely, fixation in higher dilutions of the positive serum. For this reason I am now using an amount about half as large as that recommended by Thomsen and by Thomas and Ivy.

The probable explanation of these observations is that when the dose of antigen is increased beyond a certain point, the amount of complement which has to be added to overcome its anticomplementary effect is too great to be fixed by certain grades of positive serum, or, in other words, that beyond a certain point the anticomplementary effect of antigen increases more rapidly than its antigenic effect. This suggests a method of determining the optimal dose of each new antigen for the Thomsen or Thomas and Ivy or the Browning and McKenzie methods. This method will be discussed below. The experiments on which it is based are described in a separate publication.[32]

Wassermann himself laid down the rule that the dose of antigen should be one half of the largest amount which itself was found to be not at all anticomplementary; and the tests were not to be read until the control tube containing double the dose of antigen used in the tests

32. Ottenberg: Methods of Determining the Optimum Amount of "Antigen" for the Wassermann Reaction, Jour. Immunol., to be published.

showed complete laking. It would seem hardly necessary to point out today that in doing the titration to determine the nonanticomplementary dose it is necessary to first incubate the antigen complement mixtures for the same length of time and at the same temperature as the preliminary incubation in the test proper, and only then to add sheep cells and amboceptor. With a good antigen of the simple alcoholic extract type the amount prescribed by Wassermann usually contains at least five to ten antigenic units, that is, it is at least five to ten times as large as the smallest dose which gives complete fixation with a positive serum.

The rule of one half the nonanticomplementary dose has been departed from by probably the majority of subsequent workers, apparently without a very good reason. Thus, with alcoholic extracts, MacIntosh and Fildes use one third of that amount, which alone is completely anticomplementary; Kaliski uses from one third to one fourth of a dose which shows beginning anticomplementary effect; the New York health department uses from one third to one fourth of a dose which shows no anticomplementary effect. With cholesterinated antigens likewise there is the widest divergence in the method of deciding the dose; thus Field specifies one fifth of the dose which begins to show anticomplementary power, Walker and Swift one fourth of the dose which is completely anticomplementary, Sachs a half of a dose which itself is not at all anticomplementary.

An entirely different principle is used by those who follow the method of titrating the complement (Thomsen, Boas, Thomas and Ivy, Browning and McKenzie) for each day's work in the presence of the antigen. All these workers use fixed and purely arbitrary doses of antigen, adjusting the dose of complement to that of antigen in such a way that the amout of antigen used is not one half or smaller fraction of the nonanticomplementary amount, but actually the largest nonanticomplementary dose itself; thus, double the amount of antigen used is decidedly anticomplementary. In fact, Boas states that his antigen should be completely anticomplementary in the double dose. The adjustment is not of antigen to complement, but of complement to antigen. In making this adjustment the fixed dose of antigen and varying doses of complement (and in the methods of Browning and McKenzie and of Thomas and Ivy, also pooled negative serum) have to be first incubated under precisely the same conditions as in the preliminary incubation of the test itself. The fixed dose used by Thomsen and by Boas is 0.2 c.c. of the alcoholic heart extract, that used by Stern 0.3 c.c. of the alcoholic heart extract, by Sormani from 0.25 c.c. to 0.3 c.c., by Thomas and Ivy 0.2 c.c. of alcoholic extract of syphilitic liver, by Browning and McKenzie 0.2 c.c. of cholesterinated alcoholic beef heart extract.

There does not seem to be a fixed principle on which the selection of this arbitrary dose is based except the principle of empirical experience, the fact that one obtains satisfactory results with the dose used. All of these workers used doses which, compared with those used in the original Wassermann system, are large, but the hemolytic system is adjusted to the dose of antigen. Though the larger dose superficially seems to have the advantage of providing a greater multiple of antigenic units than the smaller dose, this is not actually the case. And (as is pointed out in a separate communication) the dose should not be arbitrary, but should be determined accurately by ascertaining which one of a series of possible doses of antigen, when combined each with its appropriate dose of complement, gives complete fixation with the highest dilution of a positive serum.

To summarize this important subject of dosage of antigen, there are two schools of workers, those who adjust the dose of antigen to the complement and those who adjust the dose of complement to the antigen.

In the former school, comprising such workers as Wassermann, Citron, Sachs, Walker and Swift, Noguchi, and New York department of health, the standard by which the dose of antigen is determined is the anticomplementary effect; but the method of adjusting the dose according to the anticomplementary effect varies widely. It is suggested that in the interest of uniformity all workers who follow this plan should adhere to Wassermann's original standard, the dose of antigen which of itself, after preliminary incubation, is not at all anticomplementary. This dose, in my experience, is more constant and easily determined than the dose which begins to be anticomplementary or the dose which is just completely anticomplementary. Which particular fraction, one half, one fourth, one sixth, or what not, of this nonanticomplementary dose should be chosen, is a matter of empirical determination and has to be settled for each type of antigen separately. Probably a majority of the workers who use simple alcoholic extracts or acetone insoluble lipoids would agree on one half of the nonanticomplementary dose. With cholesterinated antigens it is generally recognized that a smaller fraction is necessary. Boas[33] pointed out in 1913 that if the usual rules of dosage were applied to cholesterinated antigen nonspecific results were frequent. It is possible that some of the unfavorable reports on cholesterinated antigens in the literature are based on dosage relatively too great.

The second school of workers, who use an arbitrary dose of antigen and adjust the dose of complement to it comprise such workers as

33. Boas: The Wassermann Reaction, Ed. 2, 1913.

Browning and McKenzie, Thomsen, Boas, Sormani, M. Stern, and Thomas and Ivy. This method seems to me at least as good as the preceding. But the dose of antigen should not be arbitrary, because for each antigen there is an optimal dose. And this optimal dose is easily determined by trial of each proposed dose with a series of dilutions of a positive serum.

The method by which the alcoholic antigen is diluted with saline solution is of some importance. Sachs and Rondoni[34] in 1909 showed that by fractional dilution, that is, by the slow addition of alcoholic antigen to saline solution, a more opaque emulsion and in some instances a stronger antigen was obtained than by rapid addition of saline to antigen or of antigen to the saline solution. In my experience, while the slow dilution sometimes does give a smaller minimal antigenic dose, that is, a smaller amount is needed to give a positive result, it also makes the antigen anticomplementary in a smaller dose, and therefore the value of the antigen (the ratio of the two) remains the same. The important thing, therefore, is not which method of dilution (slow or rapid) is used, but that whichever method is used should be adhered to uniformly throughout.

The Choice of Antigen. — The great majority of workers today believe that the aqueous and alcoholic extracts of syphilitic organs need no longer be seriously considered in the practical performance of the Wassermann reaction. There are at present three types of lipoid extracts that have received general acceptance. Although it seems quite possible that of the many other lipoid preparations which have been recommended for the Wassermann reaction some may be quite as good or even better than these three, we can limit our consideration to them, namely, to the simple alcoholic extract of heart muscle, the alcoholic extract purified by acetone precipitation after the method of Noguchi, and the alcoholic extract reinforced by cholesterin according to the recommendation of Sachs, Walker and Swift, and others.

In spite of the enormous amount of work which has been done on the subject, it is impossible to draw final conclusions as to which type of antigen is best. I have used simple alcoholic extracts of beef and of guinea-pig heart, acetone insoluble fraction of beef heart, cholesterin reinforced guinea-pig heart, and cholesterin reinforced human heart. The simple alcoholic extracts from the different species of heart muscle seem to be practically identical in action, although there are rare cases in which a serum gives a positive reaction with one species and not with another. Personally, I have come to rely on beef extract because it is the easiest to prepare and extremely uniform in titer.

Although in my hands the acetone insoluble fraction has not shown any decided superiority over the simple alcoholic extracts, I have not

34. Sachs and Rondoni: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1908-1909, **1**, 132.

used it in parallel tests extensively enough to offer a definite judgment as to its value.

The use of cholesterin reinforced alcoholic extract is a vexed question. I have used this antigen in over 1,500 tests in which at the same time the reaction was done with simple antigen. Of these, the first 250 will be disregarded, as I am inclined to consider the rather high proportion (10 per cent.) of disagreements obtained in them as due partly to my inexperience with the method used (that of C. W. Field).

In the last 1,241 tests the method used has been that followed by Walker and Swift, and I have had the invaluable assistance of Dr. E. C. Jaegle, who has been using this method for years. The only change in the method has been the addition of an extra tube containing the half dose of patient's serum and the half dose of antigen, so that the quantitative readings (four plus, three plus, two plus and one plus) could be made according to the scheme of Citron, given farther along in this paper.

If the weak inhibitions (plus and two plus) are disregarded, the two antigens gave identical results in 1,174 cases, 95 per cent. of the total. Of the sixty-seven disagreements, forty-two gave definite positive reactions (four plus and three plus) with cholesterin and negative or doubtful reactions with simple antigen. Of the forty-two, ten were cases of undoubted syphilis and ten were doubtful cases, in which syphilis could not be excluded clinically.[35] Twenty-two were cases in which there was no reason to suspect syphilis. Of this twenty-two, eighteen were cases of pregnancy (7 per cent. of the 244 pregnancy cases examined).

On the other hand, twenty-five of the sixty-seven disagreements were cases in which cholesterinated antigen gave negative, simple alcoholic extract positive results. Twenty of these were known or highly probable cases of syphilis, five were doubtful cases in which syphilis could not be excluded.

It must be confessed, however, that if weak inhibitions (plus and two plus) are taken into account, cholesterinated antigen, though it gave a far greater number of weak positive reactions in treated syphilis, also gave a considerable number of false positive reactions in cases surely not syphilitic.

From this experience I venture to draw the following conclusion: Cholesterin reinforced antigen should never be relied on alone for diagnosis; weak inhibitions with it should not be given great weight excepting in known cases of syphilis; positive results obtained with it

35. Most of the cases were ambulant and the clinical diagnoses were those of the physicians in charge of the various departments of the Vanderbilt Clinic, the Sloane Hospital for Women, and the Knapp Memorial Hospital for Eye Disease.

alone in pregnancy should be disregarded. On the other hand, in following the progress of cases of syphilis under treatment, the cholesterinated antigen is of very great value because it continues in many cases to give weak positive reactions long after the reaction to simple antigen has disappeared; and the opinion of syphilographers is that treatment should be continued until the reaction to cholesterinated antigen is also expunged. On the other hand, cholesterinated antigen should not be relied on alone in these cases either; for, as the author's experience shows, there is a not inconsiderable number of cases of known or treated syphilis in which a positive result is obtained with simple, but not with cholesterinated, antigen.

QUANTITIVE READINGS OF THE WASSERMANN REACTION

In recent years the demand has more and more been made that the result of the Wassermann test be not merely reported as positive or negative, but that the strength of a positive reaction be indicated. This is of particular value in observing the result of treatment. A few workers have satisfied themselves by merely judging the strength of the reaction from the degree of inhibition in a single tube; but it is apparent that this method is extremely inaccurate, and various methods of titrating the strength of the reaction have been proposed.

Beyond a doubt great confusion has been caused in the medical mind by the divergent systems used by different workers for discovering and reporting the strength of reactions. This is one of the chief causes of the present lack of confidence in the Wassermann reaction. For this reason, as well as for its intrinsic importance, the subject deserves special attention.

Since the Wassermann reaction is a reaction between three different substances, antigen, patient's serum, and complement, it is evident that the reaction may be reduced by progressive steps to the vanishing point by using progressively diminishing (or in the case of complement progressively increasing) amounts of any one of these three substances. As a matter of fact, all three methods, that of using diminishing amounts of patient's serum, or increasing amounts of complement, or diminishing amounts of antigen, have been proposed and used with success by different workers for this purpose.

Citron, who was one of the first to introduce quantitative methods into the practice of the Wassermann reaction, employs two of the principles, that is, he uses simultaneously descending doses of both antigen and patient's serum. He uses only two tubes, the first tube containing the full dose of antigen and serum, the second half the dose of each, and he makes readings as follows:

Tubes 1 and 2 show complete absence of hemolysis	++++	Strongly positive
Tube 1 shows complete absence of hemolysis, and Tube 2 shows faint hemolysis	+++	
Tube 1 shows complete absence of hemolysis, and Tube 2 shows complete hemolysis	++	Weakly positive
Tube 1 shows partial hemolysis, and Tube 2 shows complete hemolysis	+	
Tube 1 shows doubtful binding, and Tube 2 shows complete hemolysis	±	Doubtful
Tubes 1 and 2 show complete hemolysis		Negative

Readings made in this way have the advantage of indicating four different degrees of positive reaction with the addition of only one extra tube. And if for the time being one takes it for granted that to use half the dose of antigen gives the same result as to use half the dose of serum, the four grades may be taken to correspond roughly to four, three, two and one units of positive reaction.

It should be pointed out that there are many workers at present who in their reports use Citron's scheme of notation (four plus, three plus, two plus and one plus), but without using Citron's method of reading the strength of the reaction. This is done even by many who depend on the appearance of a single tube for determining the strength of the reaction; and this practice has led to many apparently inconsistent reports.

Thomsen and his pupil, Boas, use the method of descending doses of patient's serum; in the tubes in which there is incomplete inhibition they read the percentage of laking by comparing the color after sedimentation of the cells with the color of a hemoglobin scale (the hemoglobin scale is made by laking the same amount of red cells as used in the test, and of this laked mixture preparing successive percentage dilutions with water). They only report positive in any given tube if there is over 20 per cent. of inhibition according to this scale, and they express the strength of the reaction on a numerical scale. This method has been followed by many other workers. MacIntosh and Fildes, who also employ descending serum doses, believe that they can show that descending amounts of antigen give relatively abrupt, while descending doses of patient's serum give relatively gradual, changes. This needs confirmation.

I have used the Thomsen method (descending doses of patient's serum, 0.2 c.c., 0.1 c.c. and 0.05 c.c.) in approximately 1,000 tests, parallel with the Citron method, and have found a fairly close correspondence. Fixation with full dose, half dose and quarter dose of serum correspondingly roughly to one plus, two plus and four plus in the Citron scale.

Sormani in 1911 appears to have introduced the method of using descending doses of antigen. He uses five doses, of which the smallest is the least dose that gives complete inhibition with a very strong

secondary syphilitic serum, and the other five are successive multiples (2, 3, 4 and 5 times) of this. He reads his results 0.2, 0.4, 0.6, 0.8, 1 positive according as one, two, three, four or five of the tubes show complete inhibition. A good many other workers have adopted this method; for example, Field uses six arbitrary doses of antigen and has a rather more complicated numerical system for expressing the results.

The third method, that of using increasing units of complement, seems to have been introduced by Browning and McKenzie and has been adopted by Thomas and Ivy. As explained above, in the section on the adjustment of complement, Thomas and Ivy titrate complement in the presence of antigen and negative serum and adjust the dose in such a way that one free unit of complement is present in the main test. In successive tubes, then, they set up tests with the same amount of antigen and patient's serum and containing two, three, four or more free units of complement.

I have adopted the method of Thomas and Ivy and used it extensively. Two extra tubes are set up, the one containing two, and the other four free units of complement. It should be explained that the measuring of two and four free units of complement in these tubes is attained, of course, not by using two and four times the amount of guinea-pig serum found necessary in the first tube in the titration, but by adding to the amount in the first tube the unit of complement as determined in a separate titration (in which antigen and normal serum are not present). (See below.)

In approximately 5,000 tests I have used this method of ascending units of complement, parallel with the Citron method of descending doses of antigen and serum, and have found that one, two, three and four plus, according to the Citron method, in the great majority of cases correspond closely with the power to fix one, two, three or four units of complement in the Thomas and Ivy method.

I have also experimented with the method of Browning and McKenzie, using cholesterin reinforced antigen and ascending units of complement. From the point of view of accuracy this method is ideal, since it shows exactly how many units of complement are fixed by the reaction.

With this method anticomplementary serums cause no difficulty, since the anticomplementary effect of each serum (as well as that of the antigen) is measured directly in units of complement, and subtracted from the number of units of complement fixed in the test. For the same reason natural antisheep hemolysin plays practically no rôle because its effect in the control series is directly subtracted from the main series. I have found, however, that the amounts of complement specified in Browning and McKenzie's communications are far too

large to detect any except very strongly positive serums; and I have come to use three, five and eight units instead of seven, ten, fifteen, twenty, thirty and forty, as advised by Browning and McKenzie. The only objection to the method is that it is too elaborate for routine work with a large number of tests. In the simplified form used by me, however, it requires only six tubes for each test; on account of the superior opportunities for accuracy it offers it is worthy of a wider use than it seems to have had.

From my trial of all these methods I am convinced that the present status of the Wassermann reaction would be greatly improved if any one of these ways of making quantitative estimations could be adopted by all workers, and it would not make a great difference which one was adopted. The methods, however, which offer special advantages for standardization are those (Browning and McKenzie, Thomas and Ivy) which depend on the principle of measuring the number of units of complement fixed, that is, measuring the work of complement fixation directly in the amount of work done. I regard complete fixation of one free unit of complement with simple alcoholic antigens and of two free units with cholesterin reinforced antigens as the minimal definitely positive reaction.[36]

Since Citron's terminology, one, two, three and four plus has impressed itself deeply on the popular medical mind it had better be preserved, one plus representing the weakest definitely positive grade as defined above and four plus the strongest.

MINOR TECHNICAL CONSIDERATIONS

The strength of the saline solution used throughout the test varies in practice of different workers from 0.85 per cent. to 0.9 per cent. Probably it makes no difference which of these two strengths is used provided the same strength is adhered to uniformly. It is of real importance that the solution be made with accuracy, however, and occasionally disasters which occur in the laboratory are traced to errors in this step.

The water used is of course distilled water, and as this contains carbon dioxid absorbed from the air, it is the practice of some laboratories (New York health department) to boil the saline and then add tenth-normal potassium hydroxid solution until a sample gives a faint pink with phenolphthalein. As all the ingredients of the test are exposed to the air before and during the test, however, this seems an unnecessary refinement.

Absolute cleanliness of the glassware is a thing of great importance and occasional discrepancies and errors which occur (and which of course should be easily detected if the proper controls are used) are traced to foreign substances adhering to the tubes. The method of cleaning test tubes used at the New York health department laboratory is the best I have seen. The

36. The mode of measuring the unit of complement must of course be agreed on. I have throughout my work titrated the complement unit with two units of amboceptor and I have used this fixed dose of amboceptor in all tests. The principles laid down hold good, however, whether this particular method of titration is adopted or not.

tubes, packed firmly in copper baskets, are rinsed in tap water, and boiled in soap water; the alkali of the soap water is neutralized by dipping the baskets in dilute hydrochloric acid, after which they are immersed in running tap water and dried in ovens.

It has been claimed[37] that the alkalinity of new glassware may lead to false positive tests. This has never occurred in my experience. Nevertheless it seems wise thoroughly to boil new glassware in distilled water.

The test tubes used must be fairly uniform in caliber. Hemolysis is distinctly delayed in very narrow tubes, possibly due to imperfect mixing of the ingredients.

The use of preserved complement has the advantage that the mixed complement from a large number of guinea-pigs can always be used. Two methods of preserving complement are in vogue—salting[38] (addition of from 8 to 25 per cent. of sodium chlorid; dilution with distilled water before use) and freezing. To judge from the literature both methods are satisfactory. Stern[39] claimed that frozen complement lost some of its binding power, though not its hemolytic power; this has had no recent confirmation and should be reinvestigated. I have used salted complement and found it satisfactory. The salted complement must, however, be kept at a temperature very near 0 C. or it gradually deteriorates.

GENERAL PRECAUTIONS FOR ELIMINATION OF ERROR

It is impossible to discuss here all of the elements which enter into an accurate and safe technic, but there are a few special points, not so generally observed as they should be, that deserve special discussion. One of these is the doing of all tests as well as all titration in duplicate. The Wassermann reaction is a quantitative reaction. In its essence it is probably also a chemical reaction. No chemist would think of relying on a single quantitative determination in an important case. He would make a duplicate, and if the two determinations did not agree, he would make another estimation. Accurate results with the Wassermann test are more rather than less difficult to obtain than are accurate results in purely chemical work. For this reason, in spite of the increased labor, all titrations as well as all tests ought to be done in duplicate.

It is advisable in duplicate tests to vary the technic slightly so as to get the advantage of seeing from two different angles — binocular vision. Many workers do this by employing two or more different antigens. As has been pointed out in the foregoing, differences in results are due quite as often to variations in technic as to differences in antigen. For this reason different technics as well as different antigens should be employed, at least until some one system has been shown to be greatly superior to all others. At present I make all tests by three methods, as follows:

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37. Sternberg: Wien. klin. Wchnschr., 1914, **27,** 545.

38. Austin: Jour. Am. Med. Assn., Correspondence, 1914, **62,** 868. Thompson: The Preparation and Preservation of Complement, New Instruments and Suggestions, Jour. Am. Med. Assn., 1916, **66,** 652.

39. Stern: Berl. klin. Wchnschr., 1908, **45,** 1489.

1. A modified Thomas and Ivy method with simple alcoholic beef heart extract; preliminary incubation four hours in the icebox; use of two extra tubes with increasing doses of complement (two and four units) for determining the strength of the reaction; one extra serum control tube with two units of complement;[40] final addition of cells and two units of amboceptor.

2. A modified Citron method with simple alcoholic extract, icebox incubation, two units of complement (the unit determined with two units of amboceptor), Kaliski's device (see above) for detecting and compensating for natural antisheep hemolysin, final addition of two units of amboceptor when needed.

3. Cholesterin reinforced antigen according to the method employed by Walker and Swift, slightly modified, and with the addition of Citron's tube for determining the strength of the reaction; two units of complement in the "main" tubes, one unit in the serum control tube (the unit is determined with one unit of amboceptor and hence is larger than in the modified Citron method described above); two units of amboceptor; preliminary incubation in water bath at 37 C., and final addition of sensitized sheep cells, without attention to natural amboceptor.

Another precaution that should be taken much more often than it is, is the checking up of results with the results of another laboratory. Every laboratory should have an arrangement with at least one or two other laboratories for the regular and frequent interchange of serums on which the results can be compared.

Of even greater importance is the clinical checking up of the cases. In reading the results of the tests the laboratory worker should pay no attention to the clinical diagnosis. But after the results have been read objectively and recorded they should all and invariably be compared with the clinical diagnoses. Many clinicians do not realize the importance of this, and harbor unfair suspicions of the laboratory man when he insists on knowing the clinical diagnoses. But as a matter of fact, since the Wassermann reaction is a test which has no absolute, but only a practical, specificity, it is necessary constantly to check up the results by a knowledge of the clinical diagnoses. It sometimes happens, for instance, that with this knowledge on a particular date the laboratory worker is able to recognize the fact that all his tests are "weak," that is to say, due to some inadvertent variation in technic, such as the use of too large a dose of complement, some weakly positive serums have not been detected. This being known, he can then repeat the tests with proper precautions.

A further control on the constancy and delicacy of the reaction in detecting weak positive results is the use of a weakly positive control serum, as introduced by Citron. This consists of setting up, in addition to the usual control test with a known strong positive serum, another test, using a serum known from previous testing to be weakly positive. If no such serum is on hand, a strongly positive can be diluted four to eight times. It has to be remem-

40. This is provided in case the first serum control tube containing only one unit of complement fails to lake completely. If this happens then the first "main" tube is disregarded and readings made only from the tubes containing two and four units of complement.

bered, of course, that positive serums become weaker on standing for more than a few days unless special precautions are taken to preserve them.

The antigen control test is, of course, absolutely essential, no matter how often or how recently one has used an antigen or how great one's confidence is in its reliability. This is particularly true since a rare occurrence is the finding of a guinea-pig whose serum gives a positive Wassermann reaction. I have seen this once only in several thousand of guinea-pigs used in the last seven years. Dr. Jaegle has seen it twice.

CONCLUSION

Divergent reports on identical serums sent to different laboratories undoubtedly occur and will continue to occur so long as laboratory workers continue to use widely different technical methods. These divergent results, however, should not shake our confidence in the clinical specificity of the Wassermann reaction. They almost invariably occur only in cases which exhibit weakly positive reactions, and they usually mean that one laboratory has succeeded in detecting a weakly positive reaction, while the other has not. In the great majority of cases which present definite positive or definite negative results the reports of different laboratories are practically uniform. The reason for the divergence in results on weakly positive cases is that some laboratories have adopted certain refinements of technic which other laboratories have for various reasons failed to adopt.

The original Wassermann technic, while safe in the sense of not giving false positive results, is not nearly so delicate in detecting weakly positive tests as it can be made. Many of the devices for making the reaction more delicate without impairing its safety are discussed and recommended in the present paper.

On the other hand there are a few workers who, in their anxiety to detect as many of the weakly positive cases as possible, have adopted methods which easily can and probably do lead to occasional false positive reports.

Though the technic of the Wassermann reaction is relatively simple to learn, the work is full of pitfalls and should be done only by properly trained workers and the results should be controlled by every possible control. While it does not seem possible that all workers will adopt one uniform technic, it is greatly to be desired that they should agree on basic principles and methods.

Though the present paper has discussed the Wassermann reaction only, the observations are nearly all applicable (with due control of specificity) to complement fixation with bacterial and other specific antigens.

THE BACTERIOLOGY OF THE URINE IN FOCAL INFECTIONS; ITS RELATION TO NEPHRITIS *

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In a previous report[1] the bacteriology of the urine in a number of cases of nonsuppurative nephritis was described. In all of the cases examined the urine contained numbers of bacteria, mostly anaerobes. In one case[2] the bacterial flora of the urine was the same as that of a focus of infection in another part of the body. The bacteria isolated from the urine readily produced experimental nephritis. These facts led us to believe that bacteria multiplying in foci of infection frequently gain entrance to the blood stream and are excreted in viable condition through the kidneys, and that the excretion of bacteria in this way by the kidneys may be a common cause of nephritis.

It seemed desirable to examine a number of patients with evident focal infections to learn how frequently bacteria found in the focus of infection can be isolated from the urine, in what number they occur in the urine, and whether excretion of bacteria occurs to any extent without clinical evidence of injury to the kidneys.

In selecting cases for this study, patients with gonorrhea, other primary infections of the urinary tract, or syphilis were excluded, so far as possible, by means of history, complement deviation tests, evident discharges, pus in the urine, vesical irritation, etc. Infections such such as pneumonia, known to be accompanied by septicemia, were also excluded. No patient with evidence of an active tuberculosis was included. Some of the patients studied had evidence, such as arthritis, of migration and lodging of bacteria in other parts of the body. Cases in which the intestine seemed the most likely source of infection were avoided because of the proximity of the urethral and anal orifices, which might lead to the interpretation of correspondence between urinary and fecal flora as due to contamination rather than to transmission by the blood stream.

Eighteen patients were examined. In six of these the bacteria when found in the urine did not correspond to those found in the focus of

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* From the Memorial Institute for Infectious Diseases.

1. Dick, G. F., and G. R.: The Bacteriology of the Urine in Nonsuppurative Nephritis, Jour. Am. Med. Assn., 1915, **65**, 6.

2. Dick, G. F., and G. R.: The Bacteriology of the Urine in Two Cases of Parenchymatous Nephritis, Jour. Am. Med. Assn., 1914, **63**, 1661.

infection from which cultures were made. This may have been due to the bacteria in the urine having come from a source other than the focus of infection studied. In some instances, the negative results may have been due to the time of examination, as it was found that showers of bacteria may occur in the urine just as showers of casts are known to occur. Indeed, the two were observed to appear together in Case 5, to be described. In most cases the bacteria were hard to grow in pure culture and difficulty of technic in isolating and identifying the organisms probably explains some negative results.

In four cases a partial correspondence was found between the bacterial flora of the urine and that of the focus of infection. In eight cases the corrospondence was more striking. These eight cases will be described in detail.

Cultures from the focus of infection were made aerobically and anaerobically on human blood agar slants. Shake cultures in plain agar were made with 2 c.c. catheterized urine; 15 c.c. urine were centrifuged and aerobic and anaerobic cultures on human blood agar were made from the sediment.

REPORT OF CASES

CASE 1.—*Chronic tonsillitis, chronic interstitial nephritis, cardiac hypertrophy in a woman, aged 25.* History of frequent attacks of tonsillitis. Blood pressure 150 mm. The catheterized urine showed an occasional hyaline cast and leukocyte; no albumin or erythrocytes.

Cultures from 2 c.c. urine gave colonies too numerous to count of (*a*) streptococcus, oval, gram-positive aerobic, hemolytic, dextrose acid, mannite negative, lactose faintly acid, milk acid and not coagulated at the end of forty-eight hours; (*b*) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, no hemolysis, minute transparent colonies on human blood agar.

Cultures from the tonsils gave (*a*) streptococcus, oval, gram-positive, aerobic, hemolytic, dextrose acid, mannite negative, lactose faintly acid, milk acid, not coagulated at end of forty-eight hours; (*b*) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, no hemolysis, minute transparent colonies on human blood agar; (*c*) fusiform bacillus; (*d*) staphylococcus; (*e*) coccus, small, gram-negative, anaerobic.

CASE 2.—*Chronic rhinitis, pyelonephritis, post-operative bronchopneumonia, in a girl, aged 12.* History of "cold in head" all of preceding winter, frequent micturition for six months, tonsillectomy two months, and removal of inferior turbinate bones one week before being seen. Blood pressure 114 mm. The urine contained 0.2 to 0.8 gm. albumin to the liter; showed numerous leukocytes and some leukocytic casts, no erythrocytes.

Cultures of 2 c.c. urine gave fifteen colonies of (*a*) streptothrix, small, true branching, mostly gram-negative, growth in both aerobic and anaerobic cultures, no growth on ordinary mediums, very delicate, transparent growth of minute colonies on human blood agar, no change in the blood; one colony of (*b*) staphylococcus; one colony of (*c*) bacillus diphtheroid, gram-positive, aerobic.

Cultures of nasal secretion showed (*a*) streptothrix, small, true branching, mostly gram-negative, growth in both aerobic and anaerobic cultures, no growth

on ordinary mediums, very delicate, transparent growth on human blood agar, no change in the blood; (*b*) streptococcus, hemolytic.

CASE 3.—*Recurring bronchitis, chronic arthritis, nephritis, in a woman aged 49.* History of bronchitis every winter. Blood pressure 150 mm. Urine showed trace of albumin, occasional hyaline and finely granular cast, very few leukocytes, no erythrocytes.

Cultures of 2 c.c. urine gave one colony in shake, and several colonies on human blood agar slant, of (*a*) streptococcus, small spherical, gram-positive growth very scant both aerobically and anaerobically, forming minute, transparent colonies; hemolytic; lactose and dextrose acid, mannite negative, milk acid and coagulated; six small colonies of (*b*) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary media, minute transparent colonies on human blood agar, no change in the blood; one colony of (*c*) staphylococcus; two colonies of (*d*) bacillus diphtheroid, gram-positive, aerobic.

Cultures of sputum gave (*a*) streptococcus, small, spherical, gram-positive, growth very scant both aerobically and anaerobically, minute transparent colonies, hemolytic, lactose and dextrose acid, mannite negative, milk acid and coagulated; (*b*) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, minute, transparent colonies on human blood agar, no change in the blood; (*c*) pneumococcus.

CASE 4.—*Chronic tonsillitis, chronic interstitial nephritis, cardiac hypertrophy, in a woman aged 29.* History of tuberculosis at the age of 15; backache, headache, dizziness, dyspnea on exertion noticed during last year. No evidence of active tuberculosis; right tonsil hypertrophied, with tender swelling under angle of right jaw. Blood pressure 230 mm. Urine amounted to 870 c.c. in twenty-four hours; specific gravity 1.020; showed a trace of albumin and hyaline and granular casts.

Cultures of 2 c.c. urine gave colonies too numerous to count of (*a*) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, pin-point, transparent colonies on human blood agar, no change in the blood; (*b*) streptococcus, small, gram-positive, grew only in anaerobic blood slant and failed to grow in transfers; (*c*) leptothrix varying from short bacillary forms to long threads, gram-positive, anaerobic, three colonies obtained from 2 c.c. urine; (*d*) staphylococcus, one colony grew from 2 c.c. urine.

Cultures of tonsils showed (*a*) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, pinpoint, transparent colonies on human blood agar, no change in the blood; (*b*) streptococcus; (*c*) staphylococcus; (*d*) coccus, small, gram-negative, anaerobic; (*e*) bacillus, anaerobic, gram-positive, diphtheroid.

CASE 5.—*Chronic infection about root of an impacted tooth; chronic interstitial nephritis; cardiac hypertrophy.* History of diphtheria, scarlet fever and influenza. Complaint of headache and dizziness for one year. Blood pressure, 170 mm. Urine: 1,500 c.c. in twenty-four hours; sp. gr., 1.008; no erythrocytes or leukocytes, an occasional hyaline cast and no albumin on admission; later, a trace of albumin and a shower of hyaline and granular casts.

Cultures of 2 c.c. urine made on admission to hospital gave one colony of (*a*) bacillus, small, pleomorphic, not definitely gram-positive or gram-negative, anaerobic, no growth on ordinary mediums, pinpoint transparent colonies on human blood agar, no change in the blood.

Cultures of 2 c.c. urine made at the time the shower of casts appeared showed innumerable colonies of (*a*) bacillus obtained in first culture; (*b*) bacillus, fusiform, gram-negative, anaerobic; (*c*) coccus, very small, spherical, gram-negative, anaerobic, less growth on ordinary mediums than on human blood agar, no change in the blood, delicate growth of pinpoint transparent colonies;

(*d*) streptococcus, small, oval, gram-positive, anaerobic, hemolytic, small transparent colonies on human blood agar, dextrose and lactose acid, mannite negative, milk acid and not coagulated at end of forty-eight hours; (*e*) *Staphylococcus aureus;* (*f*) *Bacillus mucosus.*

Cultures of pus from infection around tooth showed (*a*) bacillus, small, pleomorphic, not definitely gram-positive or gram-negative, anaerobic, no growth on ordinary mediums, pinpoint transparent colonies on human blood agar, no change in the blood; (*b*) bacillus, fusiform, gram-negative, anaerobic; (*c*) coccus, very small, spherical, gram-negative, anaerobic, less growth on ordinary mediums than on human blood agar, no change in the blood, delicate growth of pinpoint transparent colonies; (*d*) streptococcus, small, oval, gram-positive, anaerobic, hemolytic, small transparent colonies on human blood agar, dextrose and lactose acid, mannite negative, milk acid and not coagulated at the end of forty-eight hours; (*e*) *Staphylococcus aureus;* (*f*) bacillus, very small, plump, gram-negative, anaerobic, hemolyzed blood slowly forming black colonies; (*g*) streptococcus, larger than (*d*) spherical, long chains, gram-positive, aerobic, heavier growth than (*d*) milk acid and coagulated at end of forty-eight hours.

TABLE GIVING DATA OF AUTHORS' CASES

Case No.	Clinical Evidence of Kidney Lesion	Organisms Found in Both Urine and Focus of Infection	Number of Bacteria in Urine
1	+	(a) Streptococcus (b) Anaerobic bacillus	Many
2	+	Streptothrix	Few
3	+	(a) Streptococcus (b) Anaerobic bacillus	Few
4	+	(a) Streptococcus (b) Anaerobic bacillus	Many
5	+	(a) Streptococcus (b) Anaerobic bacillus (c) Fusiform bacillus (d) Anaerobic coccus	Few to Many
6	−	(a) Pneumococcus (b) Streptothrix	Many
7	?	(a) Streptococcus (b) Anaerobic bacillus	Many
8	+	Leptothrix	Many

CASE 6.—*Bronchiectasis (nontuberculous), in a woman aged 44.* History of cough and expectoration for three months following an attack of influenza. Blood pressure, 120 mm. Catheterized specimen of urine showed no albumin, casts, leukocytes or erythrocytes.

Cultures of 2 c.c. urine gave numerous colonies of (*a*) pneumococcus; (*b*) streptothrix, small, very pleomorphic, forming branched threads at times, at other times appearing as a very irregular, small bacillus, gram-negative, anaerobic, no growth on ordinary mediums, delicate transparent growth on human blood agar, no change in the blood; (*c*) bacillus, one colony only, of morphology of colon bacillus, gram-negative, aerobic, not motile, thick, moist, yellowish mucoid film on plain agar, no change in blood, lactose, milk acid and slowly coagulated, mannite and dextrose negative.

Cultures of sputum showed (*a*) pneumococcus; (*b*) streptothrix, small, very pleomorphic, forming branched threads at times, at other times appearing

as a very irregular small bacillus, gram-negative, anaerobic, no growth on ordinary mediums, delicate transparent growth on human blood agar, no change in the blood; (*c*) yeast.

CASE 7.—*Exophthalmic goiter, chronic tonsillitis; mitral stenosis and insufficiency, in a woman aged 25.* History of typhoid fever. Nervousness, dizziness, palpitation, diarrhea and one recent attack of nausea during past year. Blood pressure, 150 mm. Urine showed no albumin, casts, leukocytes or erythrocytes.

Cultures of 2 c.c. urine showed numerous colonies of (*a*) bacillus, small, regular in morphology in direct smears of urinary sediment, pleomorphic in cultures, gram-negative, anaerobic, no growth on ordinary mediums, very delicate growth of pinpoint transparent colonies on human blood agar, no change in the blood; (*b*) streptococcus, marked tendency to pleomorphism with formation of long oval and bacillary forms, many of which are gram-negative, nonhemolytic.

Cultures of tonsils showed (*a*) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, very delicate growth of pinpoint transparent colonies on human blood agar, no change in the blood; (*b*) streptococcus, nonhemolytic, resembling that of urine; (*c*) streptococcus, hemolytic.

CASE 8.—*Chronic tonsillitis, acute rheumatism, aortic insufficiency, chronic nephritis, in a woman aged 25.* History of measles, whooping cough, tonsillitis, two attacks of rheumatism, dyspnea and palpitation. Blood pressure varied from 165 mm. to 190 mm. Urine showed albumin and coarse and fine granular casts, no leukocytes or erythrocytes.

Cultures of 2 c.c. urine gave about forty colonies of (*a*) leptothrix, thread-like forms, no branching, gram-positive, anaerobic, growth slow, forming minute, transparent colonies on human blood agar at end of forty-eight hours, which developed into slightly raised, brownish colonies about 1 mm. in diameter at end of five days, blood brownish, growth on dextrose, mannite lactose agar and in milk slight as compared with that on human blood agar.

Cultures of throat showed many colonies of (*a*) leptothrix of same characteristics as that of urine.

SUMMARY AND CONCLUSIONS

It was pointed out in our previous report[3] that staphylococci and gram-positive aerobic diphtheroids are common inhabitants of the urethra. Their occasional presence in small numbers as a contamination in catheterized urine is to be expected. They were found in Cases 2, 3, 4 and 5. It will be noted that aside from these organisms, one or more of the kinds of bacteria found in the urine were isolated from the focus of infection in each of the eight cases. It seems probable that the bacteria found in the urine came from the focus of infection.

It will be seen from the table that two of the eight patients showed no clinical evidence of renal lesion. One of these, however, had persistently high blood pressure (150 mm.). In the remaining patients there were from mild to severe renal lesions present, but there was no very constant relation between the severity of the lesion and the number of bacteria in the urine.

3. Loc. cit., Note 1, p. 15.

The finding of considerable numbers of bacteria in the urine in 66 per cent. of patients with evident foci of infection indicates that in diseases where migration of bacteria from foci of infection takes place, the bacteria are often present in the urine.

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